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GENETICS

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THE BAROID MUTATION IN *DROSOPHILA MELANOGASTER*

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Received October 20, 1931

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INTRODUCTION

Bar, a sex-linked semi-dominant mutation, was found by TICE (1914). Since then no more mutations from wild-type to bar have been observed, in spite of the fact that the change in the eye shape produced by bar is striking enough not to be easily overlooked. As contrasted with the scarcity of mutations from wild-type to bar, the bar gene proved to be rather unstable. MAY (1917) and ZELENY (1919) showed that bar reverts to wild-type, and also gives rise to a new, more extreme, allelomorph, double-bar (or ultra-bar). ZELENY (1920, 1921) found that double-bar reverts to bar and directly to wild-type, and that the wild-type derived from bar by reversion is as stable as a regular wild-type.

STURTEVANT and MORGAN (1923) and STURTEVANT (1925, 1928) discovered that the reversions from bar and double-bar to wild-type, as well as the changes from bar to double-bar and from double-bar to bar, occur only if crossing over takes place in the immediate neighborhood of the bar locus. The work of STURTEVANT proved that these "mutations" represent a phenomenon *sui generis*, different from the usual point-mutations. The "mutations" at the bar locus are due to unequal crossing over. Two chromosomes undergoing crossing over are normally broken at exactly the same levels, so that no gene is lost or acquired in the process of exchange.

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If crossing over takes place at the bar locus the sections exchanged may be unequal. One of the resulting chromosomes may carry both bar genes present in two original chromosomes, and the other chromosome may carry no bar. Double-bar represents two bar genes lying side by side in the same chromosome, and the reversion from bar to wild-type is due to the loss of bar. Wild-type found in nature or derived from bar by reversion has no bar allelomorph at all.

STURTEVANT (1925) found that the bar gene, once present in the chromosome, may undergo mutational changes of the usual kind. He observed a mutation, not associated with crossing over, from bar to a less extreme allelomorph, infra-bar. Infra-bar behaves like bar, in the sense that it, by unequal crossing over, reverts to wild-type and produces a more extreme allelomorph, double-infrabar. Bar-3 (STERN) and Bar-4 (BRIDGES unpublished) are weak allelomorphs derived from bar by mutations. HANSON (1928) obtained reversions from bar to wild-type by treating bar males by X-rays. The nature of HANSON's reversions is not quite clear. They may represent losses of the bar gene or mutations to very weak allelomorphs of bar. At any rate STURTEVANT's conclusions as to the origin of "mutations" at the bar locus can be considered completely established, as shown recently by L. V. MORGAN (1931).

An important question is, however, left unsolved by STURTEVANT's investigations. If a wild-type fly has no bar allelomorph, how did the bar gene arise in the first place? A new mutation from wild-type to a bar allelomorph may throw some light on this problem. The present paper is devoted to the description of such a mutation.

The author wishes to acknowledge his obligations to Doctor A. H. STURTEVANT for his suggestions and advice, and to Mr. J. BONNER and Mrs. N. P. SIVERTZEV-DOBZHANSKY, who have done a part of the routine work.

ORIGIN OF BAROID

Wild-type males from the "Oregon" strain of *Drosophila melanogaster* were treated by X-rays and were crossed to untreated females homozygous for the fourth-chromosome recessive eyeless-2 and carrying a "*ClB*" chromosome. The X chromosome designated as *ClB* contains an inverted section (MULLER 1928) preventing most of the crossing over in the X chromosome, the dominant gene bar (*B*), a recessive lethal having no visible effect when heterozygous (*l*) and several sex-linked recessive genes (s_c, *t*, *v*, s_l).

On account of the severity of the treatment (current 50 kv, 5 ma, dis-

tance 15 cm from the anticathode, duration 60 minutes, aluminum filter 1 mm thick) most of the treated males were sterile, and the rest produced only a few offspring. Females manifesting *B* (that is, carrying the *ClB* chromosome) were selected from the offspring and crossed to homozygous eyeless-2 males. There were 513 such matings made, one female per culture. In the next generation a segregation is expected in the ratio 1 Bar ♀ : 1 Bar eyeless ♀ :1 wild-type ♀ :1 eyeless ♀ :1 eyeless ♂ :1 wild-type ♂. (Males receiving the *ClB* chromosome die due to the lethal.) Among the 493 cultures that were fertile, 457 gave the expected results, 35 produced no males at all (due to the appearance of a new sex-linked lethal in the treated X chromosome), or males possessing the characters of various newly arisen mutations (cut, forked, a mutation having spread and crumpled wings). One culture (No. 11585) produced offspring consisting of bar females and males.

A more careful examination showed that only about one-half of the females hatched in this last culture had eyes similar to those normally present in heterozygous bar females. The other half of the females and all the males had eyes distinctly larger than bar, approximately intermediate in size between bar and infra-bar. It is clear that a dominant sex-linked mutation, similar in its external effects to infra-bar, appeared in this culture. This mutation is called baroid (symbol B^{bd}). The exact counts in the culture (No. 11585) were as follows:

BAROID		BAROID EYELESS		BAR		BAR EYELESS	
♀	♂	♀	♂	♀	♂	♀	♂
25	25	21	15	33	—	20	—

The appearance of baroid is certainly not due to contaminations of any sort. Baroid represents a mutant type not previously encountered. One may conclude that the baroid mutation was induced by X-rays in the gametogenesis or in a gamete of the treated male.

THE CHANGE FROM DOMINANCE TO RECESSIVENESS

Baroid males from culture No. 11585 were crossed to several unrelated females. No offspring were obtained, due to the sterility of the baroid males. Baroid females from the same culture were crossed to *y* (yellow) c_v (crossveinless) *v* (vermilion) *f* (forked) males. The results expected in the progeny of this cross are 1 baroid ♀ :1 wild-type ♀ :1 baroid ♂ :1 wild-type ♂. There were obtained 168 wild-type ♀, 76 baroid ♂, and 66 wild-type ♂. There were no females showing any trace of baroid. The baroid males had eyes distinctly narrower than wild-type but not as

narrow as baroid males from the culture No. 11585. Baroid males were sterile. Wild-type females were crossed in individual cultures to $y\ c_v\ v\ f$ males. In one-half of the cultures half of the sons were baroid, but no baroid females were observed.

There is apparently no escape from the conclusion that baroid, which appeared as a dominant mutation, changed its behavior into that of a sex-linked recessive gene. Various attempts were made to obtain a solution of this puzzle. The simplest supposition is that baroid behaves as a dominant in flies heterozygous or homozygous for eyeless. Repeated outcrosses of females heterozygous for baroid to eyeless males failed to produce any females manifesting even traces of baroid. Females heterozygous for baroid were out-crossed to males from several other stocks of different origin and genetic constitution. No baroid females were seen.

One may suppose that the flies hatched in culture No. 11585 developed under some peculiar external conditions, or that they possessed some set of genetic modifiers which made baroid behave as a dominant. Otherwise we have here to deal with some unknown phenomenon.

DESCRIPTION OF BAROID

The stock of baroid is kept by crossing females heterozygous for baroid and for the sex-linked recessives forked and fused to forked fused males. The progeny of this cross consists of wild-type females (heterozygous for B^{bd}) and $f\ f_u$ females, baroid males, and $f\ f_u$ males. Since $f\ f_u$ females and baroid males are sterile, the stock is kept without selection.

The manifestation of baroid, like that of bar and infra-bar, is subject to considerable fluctuations due to environmental and genetic modifiers. Baroid males hatching first in the cultures (at 26°) have eyes intermediate in size between those of infra-bar and of wild-type, closer, however, to infra-bar. The surface of the eye is rough and the ommatidia are strongly disarranged. The eyes of the baroid males become larger with progressing age of the culture. Males hatching very late have eyes nearly as large as wild-type. Nevertheless, baroid males can always be separated from wild-type males, due to the persistence of at least some roughening of the eye-surface and some disarrangement of the ommatidia. Baroid males are sterile. No study of the effect of temperature on the size of the eyes in baroid was made. It seems, however, that in cultures developed at 19° baroid males have smaller eyes than in cultures developed at 26°. This fact is of some interest since according to SEYSTER (1919), KRAFKA (1920), A. H. HERSH (1924), and R. K. HERSH (1924) the size of the eyes in bar,

double-bar, wild-type and their heterozygotes decreases with the rise of temperature, while in infra-bar (LUCE 1926) the opposite relation is found.

Females heterozygous for baroid are generally not distinguishable from wild-type females. In some cultures a few females were found having a very slight disarrangement of the rows of the ommatidia. Such females were tested and were found to be heterozygous for B^{bd}. The occurrence of such females is, however, erratic. Baroid can be described as a practically completely recessive gene.

ALLELOMORPHISM TESTS

The similarity of the external effects of baroid and bar leads to the supposition that they are allelomorphic. Further evidence in favor of this supposition is derived from two sources: (a) the behavior of baroid and bar in compounds, (b) the apparent identity of their loci.

Females heterozygous for B^{bd} and for f and f_u (of the constitution $B^{bd}/f\ f_u$) were crossed to forked bar males. In the next generation half of the females were forked ($B\ f/f\ f_u$), and the other half of the females were non-forked (B^{bd}/B). The size of the eyes in forked females was larger on the average than in their non-forked sibs. Due to the variability of the eye-size there was some overlapping between the two classes.

The crosses of $B^{bd}/f\ f_u$ females to forked double-bar, forked double-infra-bar, and to forked infra-bar females produce the following results. Very little, if any, difference in the size of the eyes is apparent between $B^{bd}/f\ BB$ and $f\ f_u/f\ BB$ females. A distinct difference exists between $B^{bd}/f\ B^i\ B^i$ and $f\ f_u/f\ B^i\ B^i$ flies. The former have smaller eyes than the latter. This difference is of the same order of magnitude as that between $B^{bd}/f\ B$ and $f\ f_u/f\ B$ flies. The eyes in $B^{bd}/f\ B^i$ females are considerably smaller than in $f\ f_u/f\ B^i$ ones. There is practically no overlapping between the two classes and they can be separated from each other by the size of the eyes only, without use of the forked character. The same is true of the B^{bd}/B^3 *versus* $f\ f_u/B^3$ classes; the former have smaller eyes than the latter.

Bar-4 is a very weak allelomorph of Bar. Bar-4 males have only slightly smaller eyes than wild-type males, but the eyes are provided with small emarginations not present either in bar or in baroid. Females heterozygous for B^4 also show the presence of this emargination; they can be distinguished from wild-type. (Doctor C. B. Bridges, who recently discovered B^4, very kindly permitted the writer to use this new and interesting bar allelomorph in his experiments.) $B^{bd}/f\ f_u$ females were crossed to B^4 males. Females which appeared in the next generation were separated into two lots according to their eye size. Ten females taken at random from each lot

were tested individually. Those with smaller eyes proved to be heterozygous for baroid; those with larger eyes were heterozygous for $f\, f_u$. Wild-type forked males obtained from $f\, B$ by reversion were crossed to $B^{bd}/f\, f_u$ females. All the females in the offspring had wild-type eyes.

One may conclude that baroid and at least some of the known allelomorphs of bar reinforce each other in compounds. The weaker the bar-allelomorph tested, the more evident is the reinforcing effect of baroid. No such effect is apparent on the strongest of the known allelomorphs of bar—double bar. The effect on bar and double-infrabar is noticeable, but the classes carrying and not carrying baroid overlap each other in eye size. The effect of baroid on infra-bar, Bar-3 and Bar-4 is quite pronounced. Baroid and bar act as allelomorphs.

This evidence in favor of regarding bar and baroid as allelomorphic is, however, not entirely conclusive. It is conceivable that these genes are not allelomorphic, but, because of the similarity of the developmental effects, heterozygous baroid acts as an enhancer of bar. To test this possibility the effect of heterozygosity for baroid on the manifestation of several genes decreasing the eye size was studied. The genes used were: (*a*) the sex-linked recessive small-eye (small-eye is located 1.3 units to the right of forked; this figure is based on 2386 flies), (*b*) the second-chromosome dominants Lobe and Lobe-c, (*c*) the third-chromosome dominant Deformed, and (*d*) the recessive kidney. Females heterozygous for baroid and for small-eye or kidney are not distinguishable from wild-type. No effect was noticed when flies heterozygous for Lobe, Lobe-c or Deformed were heterozygous to baroid; that is to say the corresponding classes carrying and free from baroid were seemingly alike. The same is true for flies homozygous for kidney, although in baroid males homozygous for kidney the kidney character is exaggerated.

According to SCHULTZ (unpublished) flies heterozygous for bar and for the second-chromosome dominant Star have smaller and rougher eyes than might be expected by a summation of effects of these genes. Bar and Star behave as disproportional modifiers (BRIDGES 1919). The interaction of baroid and Star was studied. Star baroid males have very narrow eyes with the ommatidia disarranged in a manner similar to that found in the sex-linked mutant lozenge. Star females heterozygous for baroid are little, if at all, different from Star females free from B^{bd}. The behavior of B and B^{bd} in respect to Star is, thus, similar. BRIDGES (unpublished) found an autosomal gene having no effect by itself but intensifying Star. The presence of this gene without Star has no effect either on B or on B^{bd}.

A duplication arose in an X-ray experiment of the writer. This duplication (unpublished) represents a section of the X chromosome attached to the third chromosome. The presence of this duplication acts as an incomplete suppressor of forked and rudimentary, indicating that the section of the X chromosome attached to the third chromosome carries the normal allelomorphs of the genes mentioned. Flies heterozygous or homozygous for bar and carrying the duplication have quite distinctly smaller eyes than the corresponding classes of flies free from the duplication. The duplication suppresses completely the effect of B^{bd} on eye-size when present in baroid males. Baroid males carrying the duplication, are, however, as sterile as baroid males free from the duplication.

THE LOCUS OF BAROID

Females heterozygous for B^{bd} and for the sex-linked recessives y, c_v, v and f were crossed to $y\ c_v\ v\ f$ males. In another experiment females heterozygous for B^{bd} and the recessives g and c_r were crossed to $g\ c_r$ males. The results of these crosses are summarized in tables 8 and 12. The frequency of crossing over between the genes involved is calculated from the data in tables 8 and 12, and shown in tables 1 and 2. The standard frequency of crossing over in the respective intervals is given for comparison (according to MORGAN, BRIDGES, STURTEVANT 1925, the locus of c_r according to MULLER 1930).

TABLE 1

The frequency of crossing over in the X chromosome.

INTERVAL	OBSERVED	STANDARD	DIFFERENCE
$y-c_v$	11.5	13.7	− 2.2
c_v-v	17.0	19.3	− 2.3
$v-f$	9.4	23.5	−14.1
$f-B^{bd}$	0.1	?	?

TABLE 2

The locus of baroid with respect to garnet and carnation.

INTERVAL	OBSERVED	STANDARD	DIFFERENCE
$g-B^{bd}$	5.7	21.1	−14.8
$B^{bd}-c_r$	0.6		

One may conclude that baroid is located between f and c_r, probably closer to the former than to the latter. The frequency of crossing over in

the X chromosome carrying baroid compared with the standard is considerably reduced, at least in the region lying to the left of *v*. This fact is suggestive, since point-mutations not associated with rearrangements of chromosomal material only very rarely produce appreciable changes in the frequency of crossing over.

A more exact determination of the locus of B^{bd} was secured in a cross involving *f* and f_u (tables 9 and 3).

TABLE 3

The location of baroid with respect to forked and fused.

INTERVAL	OBSERVED	STANDARD	DIFFERENCE
$f-B^{bd}$	0.02	2.70	−2.53
$B^{bd}-f_u$	0.15		

Baroid lies between *f* and f_u. The relative distances between B^{bd} and *f* on the one hand, and between B^{bd} and f_u on the other, indicate that baroid lies at or very near the locus occupied by bar. This conclusion is further indicated by the data presented in tables 4, 10 and 11.

TABLE 4

The location of baroid with respect to forked, bar, and fused.

INTERVAL	OBSERVED	STANDARD	DIFFERENCE
$f-(B^{bd}, B)$	0.03	0.20	−0.17
$(B^{bd}, B)-f_u$	0.16	2.50	−2.34

Baroid seems to be *unilocal* with bar. The frequency of crossing over between *f* and f_u is reduced as compared with the standard (for the standard frequency see STURTEVANT 1925).

All the $f\ B^{bd}$ and the $B^{bd}\ f_u$ males shown in tables 9, 10 and 11 were tested to determine whether any of them were fertile. All were sterile. The sterility of the baroid males depends upon the same locus which produces the eye-effect, or a locus absolutely linked with it.

UNEQUAL CROSSING OVER IN THE PRESENCE OF BAROID

Care was taken not to overlook any fly the appearance of which might have been due to unequal crossing over between the chromosome carrying baroid and that carrying bar. Among the flies obtained in the progeny of the cross $B^{bd}/f\ BB\ f_u\ ♀ \times f\ f_u\ ♂$ (table 10) there was one forked male which had eyes larger than those generally possessed by *B B* males, but smaller than

the eyes of the *B* males. The right eye of this male had 58, and the left eye had 60 facets. According to STURTEVANT (1925) the mean value for the facet number in *BB* males is equal to 29.02 ± 0.17 ($\sigma = 4.02 \pm 0.12$), and the mean value for bar males is 91.03 ± 1.76 ($\sigma = 16.61 \pm 1.25$).

It is possible that the appearance of this male was due to unequal crossing over at the bar locus, which separated the two elements of double-bar and brought one of them to lie in the chromosome carrying baroid. That is to say, the possible structure of this male is *f* B^{bd} *B*. This male was sterile—some additional evidence in favor of the assumption that it carried B^{bd}. Such an interpretation is, however, not necessary. This male may have been an extreme variant of double-bar. Its sterility may have been accidental.

THE TRANSLOCATION ASSOCIATED WITH BAROID

The reduction of the frequency of crossing over in the X chromosome carrying baroid suggests that this chromosome is involved in some kind of chromosomal aberration. A translocation may be associated with baroid. Translocations produce linkage of genes located in the chromosomes involved. Females heterozygous for B^{bd}, for the second-chromosome dominant curly (C_y), and for the third-chromosome dominant hairless (*H*) were crossed to wild-type males. The results are shown in table 5.

TABLE 5

$B^{bd}/+ \quad +/C_y \quad +/H$ ♀ × *wild-type* ♂.

MALES ONLY							
Wild-type	B^{bd}	C_y	$B^{bd}\ C_y$	*H*	$B^{bd}\ H$	$C_y\ H$	$B^{bd}\ C_y\ H$
4	34	45	2	2	41	54	1

Baroid is freely recombined with *H*, but most of the baroid flies are non-C_y, and most of the non-baroid flies carry C_y. There exists a linkage between the genes located in the X and in the second chromosomes and hence baroid is associated with an X-II translocation.

Several types of X-II translocation may occur. A section of the X may be broken off and become attached to the second chromosome; a section of II may be attached to the intact X chromosome; both X and II may be broken, each into two fragments, and these fragments may reunite to form two new chromosomes (reciprocal translocation). In any of these cases a linkage between X and II may be produced. The further analysis of the translocation must reveal the position of the "loci of the translocation" in the X and in the second chromosome. The "loci of the transloca-

tion" are the loci at which the chromosome is broken, or at which the attachment of a fragment of a different chromosome takes place. The technique of the determination of the loci of translocations is sufficiently worked out (MULLER and PAINTER 1929, DOBZHANSKY 1929a, 1930a). It is based on a simple principle. If chromosomes A and B are involved in a translocation, then the gene of the linkage-group A which exhibits the strongest linkage toward the genes of the linkage-group B lies closest to the locus of the translocation in A, and *vice versa*.

Females heterozygous for B^{bd}, f, BB, f_u, and for the second-chromosome dominants C_y and L were crossed to $f\, f_u$ males. In another experiment females heterozygous for B^{bd}, g, c_r, C_y and L were crossed to g, c_r males. In both experiments the second chromosome carrying C_y and L carried also the inverted sections C_{IIL} and C_{IIR} preventing most of the crossing over in the second chromosome. The results are presented in tables 11 and 12. In both experiments all the $C_y\, L$ flies obtained are non-baroid, and all the baroid flies are not $C_y\, L$. A glance at the tables will show that this is not true in respect to f or f_u, nor to g or c_r. One must conclude that there is no crossing over between baroid and the locus of the translocation in the X chromosome. The loci of baroid and of the translocation are apparently identical. That is to say, baroid lies at the locus at which the X chromosome is broken, or at which a fragment of the second chromosome is attached to the X. Such a coincidence of the locus of a mutation with the locus of a breakage is suggestive.

THE LOCUS OF THE TRANSLOCATION IN THE SECOND CHROMOSOME

The locus of the translocation in the second chromosome can be determined by a method similar to that used for the determination of its locus in the X. The locus in the second chromosome which shows an absolute linkage with the locus of the translocation in the X is the locus sought for. Since baroid lies at the locus of the breakage in the X, the problem is simplified. For baroid must have an apparent locus also in the second chromosome, and to determine the position of this apparent locus is tantamount to determining the locus of the translocation in II.

Females heterozygous for baroid, and for the second-chromosome recessives $a_l\, d_p\, b\, p_r\, c\, p_x$ and s_p were crossed to $f\, a_l\, d_p\, b\, p_r\, c\, p_x\, s_p$ males. The sex-linked gene f is used here to make the non-disjunctional sons distinguishable from the regulars. The results of this cross are summarized in tables 6 and 13.

The locus of the translocation (B^{bd}) lies in the $p_r - c$ interval, closer to c than to p_r. The frequency of crossing over in the intervals lying to the

TABLE 6

The frequency of crossing over in the second chromosome in the presence of baroid.

INTERVAL	OBSERVED	CONTROL	DIFFERENCE
$a_l - d_p$	13.6	13.6	0.0
$d_p - b$	30.9	31.0	− 0.1
$b - p_r$	9.0	8.5	+ 0.5
$p_r - B^{bd}$	6.5	21.3	−13.2
$B^{bd} - c$	1.6		
$c - p_z$	21.8	23.8	− 2.0
$p_z - s_p$	5.5	7.1	− 1.6

right of p_r is lower than in the control experiment (for the control data see DOBZHANSKY 1932). The most striking reduction of crossing over is observed in the $p_r - c$ interval, that is to say in the interval in which the locus of the translocation lies. It is known (DOBZHANSKY 1930b, DOBZHANSKY and STURTEVANT 1931) that the insertion of the spindle fibre in the second chromosome lies slightly to the right of p_r. The baroid translocation causes, therefore, a reduction of crossing over in the right limb of the chromosome (in which the locus of the translocation lies); crossing over in the left limb is not affected. This is a typical effect of a translocation on the frequency of crossing over in a V-shaped autosome (DOBZHANSKY 1931).

For a more exact determination of the locus of the translocation females heterozygous for baroid, and for the second-chromosome recessives *b* and v_g (vestigial) were crossed to *b* v_g males. The results of this experiment (2454 flies counted) are presented in table 7.

TABLE 7

The locus of the translocation with respect to black and vestigial.

INTERVAL	OBSERVED	STANDARD	DIFFERENCE
$b - B^{bd}$	10.6	18.5	− 7.8
$B^{bd} - v_g$	0.1		

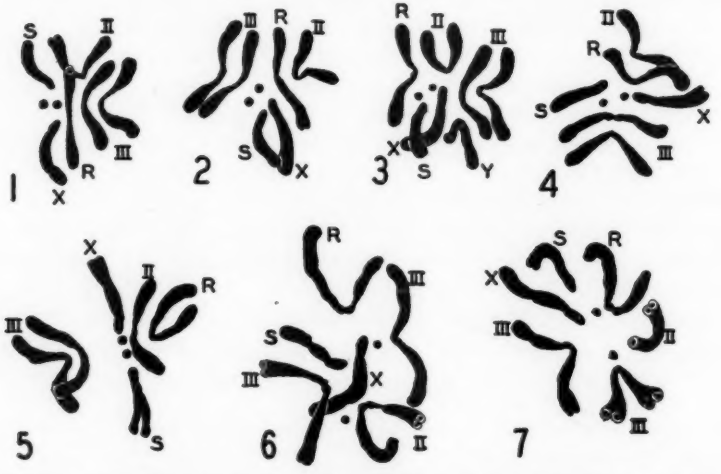
The locus of the translocation lies very close to the left of vestigial.

CYTOLOGY OF BAROID

Wild-type daughters coming from the cross $B^{bd}/f\ f_u$ ♀ × $f\ f_u$ ♂ were dissected, and their ovaries were fixed in the strong Navashin's fluid. The nerve ganglia of the larvae coming from the same cross were treated in a similar manner. A series of chromosome-plates was secured. Some of them are reproduced in figures 1–7.

The third chromosomes (figures 1–7, III), the fourth chromosomes, one of the second chromosomes (II), and one of the X's (X) appear to be normal. The other X chromosome (S) is obviously shorter than its mate. Its length is approximately three-quarters that of the normal X. The chromosome (R) which appears to be the partner of the normal second chromosome is longer than the latter. The difference between the two X chromosomes is approximately as great, or somewhat greater, than the difference between the two seconds.

The fact that the X chromosome involved in the translocation (S) is shorter than its mate proves that a section of the X is transposed onto



FIGURES 1–7.—Chromosomes of females heterozygous for baroid. Figures 1–4.—Oögonial metaphase plates; 5–7 metaphase plates from larval nerve-cells. X—the normal X chromosome; S—the X chromosome involved in the translocation; II—the normal second chromosome; R—the second chromosome involved in the translocation; III—the normal third chromosome.

the second chromosome. It is somewhat more difficult to prove that the II involved in the translocation is also broken. It is known (see above) that the locus of the translocation in the second chromosome lies near vestigial. The cytological locus of vestigial must be looked for somewhere not far from the middle of the length of the right limb of the second chromosome (for the cytological map of the second chromosome see DOBZHANSKY 1930b and 1932). If the section of the X chromosome were attached to the unbroken second chromosome at vestigial, the resulting chromosome might have, perhaps, one limb thicker than the other, but a

difference in length between it and the normal II similar in extent to that actually observed could hardly be produced.

The most probable interpretation of the nature of this translocation is represented graphically in figure 8. It is a reciprocal translocation. The X is broken at bar, and the section carrying the block of genes from s_c to f is attached to the $a_l - c_n$ section of the second chromosome. The II is broken near v_g, and the $v_g - s_p$ section is attached to the $f_u - bb$ part of the X. The distribution of the constrictions in the chromosome shown in figure 6 S is further evidence in favor of this interpretation. The chromosome referred to shows quite clearly two constrictions. One of these, the proximal one (the proximal end of the chromosome is directed toward the

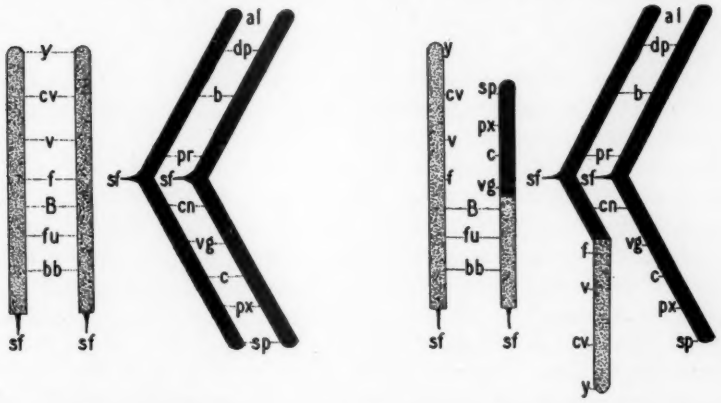


FIGURE 8.—Diagrammatic representation of the structure of the X and the second chromosome in normal females (left) and in females heterozygous for baroid (right). sf—the locus of the spindle-fibre insertion. Black—the material normally located in the second chromosome; dotted—the material normally located in the X chromosome.

center of the plate) is homologous to the proximal constriction found in the normal X chromosome (BRIDGES 1927; see also the normal X chromosome in figures 5, 6, 7). The second constriction can not be homologous to the distal constriction in the normal X, because it lies too close to the proximal constriction. This, evidently, is a new constriction marking the point of attachment of the fragment of the second chromosome to the X.

The diagram shown in figure 8 requires two comments. First, it is impossible to determine whether baroid (and bar) lie in the section of the X retaining its own spindle fibre (as it is arbitrarily shown in figure 8), or in the section attached to the second chromosome. On the face value of

the available crossing-over data one might even suppose that baroid is a second chromosome, rather than a sex-linked, gene. This interpretation is improbable in view of the fact that baroid behaves as an allelomorph of bar. Second, the occurrence of single crossovers between y and f, and between v_g and s_p, proves that the loci of the breakages and the loci of the attachments are the same (that is to say, the $y-f$ fragment is attached to the second chromosome by the f end and not by the y end, and the v_g-s_p fragment is attached by the v_g end and not by the s_p end). If the reverse were the case only doubles could be recovered.

INFLUENCE OF BAROID ON CHROMOSOME CONJUGATION

Some facts were discovered during study of the behavior of the translocation associated with baroid which are interesting because of their bearing on the problems of chromosome conjugation. These facts may be pointed at here without going into a discussion of their significance, since a discussion of essentially similar facts can be found in other papers of the author (DOBZHANSKY 1931, 1932).

Crosses involving baroid produce a considerable number of flies, the appearance of which is due to non-disjunction of the X chromosomes. For instance, in the cross of $B^{bd}/f\ BB\ f_u$ ♀ $\times f\ f_u$ ♂ (table 10) there was observed 486 $f\ f_u$ males (among 9178 males). The $f\ f_u$ males are non-disjunctional. Their frequency is 5.3 percent of the total number of male offspring. The normal frequency of primary non-disjunction is about 0.1 percent (MORGAN, BRIDGES, STURTEVANT 1925, p. 113). The increase of the frequency of non-disjunction produced by baroid is paralleled in most translocations involving the X chromosome (ANDERSON 1929, unpublished data of STURTEVANT). (Non-disjunction in baroid was studied in detail by Doctor DUNCAN. His paper will give a comprehensive account of the situation.)

The inverted sections $C_{II\ L}$ and $C_{II\ R}$ inhibit most of the crossing over in the second chromosome. In a fly possessing the $C_y\ C_{II\ L}\ L\ C_{II\ R}$ chromosome and a normal second chromosome, there is observed 0.43 percent of crossing over between C_y and L (the figure based on 3072 flies). Most of this crossing over takes place in the vicinity of the locus of p_r, which lies between C_y and L, and near which the attachment of the spindle fibre is located (see above). The presence of the translocation associated with baroid also reduces the frequency of crossing over in the second chromosome (tables 6 and 7), although not as strongly as do inverted sections. If, however, a female is heterozygous both for baroid and for the inversions, there is observed between 2.1 percent and 5.5 percent of crossing

over between C_v and L (tables 11 and 12). The interaction of two factors each of which reduces the frequency of crossing over results in an increase of the frequency of crossing over. This phenomenon was studied in more detail in the translocations involving the second and the Y chromosome (DOBZHANSKY 1932). It was found that if the locus of breakage in a translocation coincides more or less closely with one of the ends of the inverted section, the reduction of crossing over produced by the inversion is rendered ineffective in the region lying between the spindle fibre and the locus of breakage, or between the locus of breakage and the end of the chromosome.

In flies heterozygous for baroid, g, C_{IIL}, and C_{IIR}, there is observed 5.7 percent of crossing over between g and B^{bd} (table 2). DUNCAN (unpublished) found that in flies heterozygous for B^{bd} and g, but not carrying the inversions, there is less than 1 percent of crossing over between g and B^{bd}. The presence of the inversions in the second chromosome increases the frequency of crossing over in the X. A similar phenomenon has been observed previously in II–III translocations (DOBZHANSKY and STURTEVANT 1931).

DISCUSSION OF THE RESULTS

Baroid, a new mutation from wild-type to an allelomorph of bar, arose concomitantly with an X-II translocation. Baroid lies at the locus at which the X chromosome is broken, and at which a fragment of the second chromosome is attached. If such a coincidence of the locus of a breakage with the locus of a mutation had been observed only in baroid it might have been reasonably attributed to chance. In fact, however, such a coincidence seems to be far too frequent to be accounted for by mere chance.

Rearrangement of chromosomal material need not necessarily produce any effect on the characters of the organism. An individual carrying a translocation or an inversion should have, theoretically, the same genes, qualitatively as well as quantitatively, as an individual with "normal" chromosomes. The behavior of the "Pale" translocation, the first translocation observed in Drosophila, is incompatible with this theoretical expectation (BRIDGES 1923, MORGAN, BRIDGES, STURTEVANT 1925). This translocation is lethal when homozygous. In heterozygous condition it produces a dilution of the eosin eye color. The numerous translocations more recently found in Drosophila (MULLER and ALTENBURG 1930, DOBZHANSKY 1929b, 1930a, DOBZHANSKY and STURTEVANT 1931) are mostly lethal when homozygous, the lethals being, as far as known, localized usually in the vicinity of the breakage points. Some translocations are not lethal, but the homozygotes are almost always weak, sterile, or pos-

sess visible abnormalities. Individuals heterozygous for translocations are usually normal, but a number of exceptions to this rule are known. Breakages of the chromosomes are likely to produce some changes in the germplasm similar to mutational changes.

BRIDGES, MULLER and ALTENBURG, and DOBZHANSKY pointed out several possible causes of this phenomenon. Breakages of chromosomes may be due to injuries, destructions or losses of certain portions of chromosomes. Breakages themselves may produce such injuries or destructions. If so, individuals carrying translocations are *ipso facto* carrying deficiencies for certain genes. It is, furthermore, possible that mutations are for some unknown reason more likely to occur in chromosomes which have undergone breakage than in normal chromosomes. MULLER and ALTENBURG suggested also that the action of a gene may be changed by "the alteration in intergenic contiguities." A translocation, bringing about intimate contacts between genes which were not in contact before, may change the developmental effects of these genes. This suggestion is based on STURTEVANT's discovery (1925) of the "position effect" in bar.

STERN (STERN and OGURA 1931) found several translocations involving the X and the Y chromosome and accompanied by the appearance of somatic effects. The somatic effects are due to mutations from the wild-type allelomorph of bobbed located in the Y chromosome toward a bobbed allelomorph. STERN's cases are of especial value because it was possible to determine exactly which gene is affected.

A still more interesting case is described in a preliminary form by GERSHENSON (1930). An inversion of a section of the X chromosome discovered by MULLER (1928) is accompanied by a recessive lethal effect. The lethal effect arose concomitantly with the inversion; the locus of the lethal lies apparently in the vicinity of one of the two ends of the inversion. GERSHENSON observed a "reinversion," that is to say, a restoration of the normal sequence of genes in the chromosome. The lethal effect disappeared simultaneously with the reinversion. This fact proves, according to GERSHENSON, that the appearance of a lethal associated with the inversion is a reversible process, functionally related to the alteration in the gene sequence.

As mentioned above, the mutations taking place at the loci of breakages may be due to the destruction or loss of genes. This interpretation is as simple as it is difficult to test experimentally. Even explanations of the nature of point-mutations in general, based on the presence-absence notion, are revived from time to time. Baroid is perhaps the only case to which it is difficult to apply explanations of this kind. STURTEVANT (1925,

1928) proved that wild-type has no bar allelomorph. A mutation from wild-type to bar is, consequently, an addition rather than a loss. A hypothesis based on the position effect phenomenon (STURTEVANT 1925, 1928) seems, on the other hand, to lead to no difficulty when applied to baroid, as well as to GERSHENSON'S case. This hypothesis fits also the available data (meager as they are) on the "mutations" associated with the loci of breakages in translocations.

The idea that the functioning of a gene may depend upon its position in the chromosome was first advanced and proved by STURTEVANT (1925, 1928). Two bar genes lying side by side in the same chromosome (in double bar) produce a stronger effect than the same two genes do when lying in homologous chromosomes. For the present purposes the position effect hypothesis may be defined as follows: the effect of a gene on development may depend upon the properties of its neighbors as well as upon its intrinsic properties.

The problem of the origin of baroid is inextricably connected with that of the origin of bar. STURTEVANT (1925, 1928) proved that wild-type has no allelomorph of bar, but left open the question of the origin of bar. WRIGHT (1929) suggested that the original bar mutation was due to a translocation of a fragment of some chromosome onto the X chromosome. The bar "gene" may be in effect a duplicating section of a chromosome attached at the locus which we call the bar locus. THOMPSON (1931) assumes that the bar gene consists of "a main particle firmly anchored in the chromosome with varying numbers of one or more kinds of other particles attached. The main particle is called the protosome and the attached particles the episomes." The wild-type (or a reversion) possess the protosome only, bar has one protosome and one episome, and double-bar has one protosome and two episomes.

The present writer prefers to regard the "protosome" as a regular X chromosome gene, and the "episomes" as the translocated sections, carrying one or more genes, attached to or at the protosome. The expressions "protosome" and "episome" are used here and in the following text as a matter of convenience. THOMPSON (1931) attached to these expressions a definite meaning (the "side-chain theory") which is not at all what is implied in the present discussion. It is not necessary to assume that the original mutation from wild-type to bar observed by TICE (1914) involved any change in the structure of any gene located either in the X chromosome or in the attached section. The bar characteristics may be entirely due to the position effect, that is to say to the interaction between the gene lying in the X chromosome (the protosome) and the gene (or genes) located in

the attached section. The removal of the attached section by unequal crossing over (STURTEVANT) or by an X-ray treatment (HANSON 1928) causes a reversion to wild-type, just as the reinversion causes the disappearance of the lethal effect associated with the inversion (GERSHENSON 1930). The mutations from bar to infra-bar must be due, as pointed out by THOMPSON, to a change in the structure of a gene located in the attached section and not in the protosome since the reversion from infra-bar to wild-type is indistinguishable from the reversion from bar to wild-type.

The origin of baroid may, then, be due also to a position effect. The bar "protosome" is present in all flies. It is located in the X chromosome, presumably between the genes forked and small-eye. It is capable of producing the bar characteristics when subjected to the position effect of the genes located in the section attached to it in the case of bar. In baroid the X chromosome is broken at or very near the protosome. The normally existing association between the protosome and the genes lying to one side of it is removed. A new association with some of the second-chromosome genes lying normally at vestigial is established. The effect of this new association or of the discontinuation of the old ones, is the appearance of the baroid characteristics.

The assumption of the existence of a gene in the X chromosome responsible, in association with other genes, for the appearance of the bar and baroid characteristics is strengthened by the observed suppression of these characteristics by a duplication (see above). The duplication represents a section of the X chromosome carrying the loci of forked and rudimentary. If there is no gene in the X chromosome taking part in the production of the baroid characteristics and present in this duplication, the duplication need not affect baroid. The effect of the duplication may be due either to the presence of the bar "protosome," or to the altered genic balance. If the latter possibility is true one must assume that the section of the X chromosome extending from rudimentary to forked carries some loci acting as minus-modifiers of bar and baroid. Such an assumption is hardly compatible with the findings of BRIDGES (MORGAN, BRIDGES, STURTEVANT 1925, p. 165). BRIDGES studied a deficiency covering forked and bar. A fly heterozygous for the deficiency and for bar shows no exaggeration of bar. An exaggeration of bar must follow if the deficient section carries minus modifiers of bar. The only escape from the necessity of assuming the existence of the bar "protosome" is, then, to suppose that the hypothetical minus-modifiers of bar are located near rudimentary. (It would be evidently a misuse of terms to call the bar "protosome" a normal allelomorph of bar. The bar mutation is due not to the presence of the proto-

some as such but to its interaction with the genes lying in the "episome." The presence of the latter, and not of the former, constitutes the distinction between bar and not-bar.)

The result of the interaction of two adjacent genes may, obviously, depend upon the structure of either of them. The same gene ("protosome") produces bar, a dominant character, when associated with the gene (or genes) located in the bar "episome," and produces baroid, a recessive character, when affected by the proximity of some gene normally located in the second chromosome. The problem of the position effect is open to experimental attack. Translocations involving breakages of the X chromosome near the bar locus may furnish material for the solution of many questions. The question of the necessary proximity between the "protosome" and "episome" is perhaps the most important. Is the bar protosome able to produce bar-like characteristics only if the breakage takes place at its locus, or does a breakage of the X chromosome located at a certain distance from it also provoke a position effect? How great can be the differences in the effect of different "episomes" on the same "protosome"? Does the bar "protosome" affect the genes with which it is associated?

It is possible that the sterility of the baroid males depends upon a change in the developmental effect of the second-chromosome gene with which the bar "protosome" is associated. An argument in favor of this supposition is supplied by the non-suppression of the sterility of baroid males by the duplication which is known to suppress the eye-effect of baroid (see above). If this supposition is accepted the distinction between the "protosome" and the "episome" becomes entirely conventional.

The current theory of the nature of the germ-plasm is a corpuscular theory. The germ-plasm consists of a definite number of discrete units or genes, and is, consequently, essentially discontinuous. If further investigations will show that many or even all genes may show a position effect, it will be necessary to recognize that the germ-plasm represents a continuity of a higher order.

SUMMARY

1. A mutation similar in external effect to bar arose in an X chromosome of a wild-type male treated by X-rays. The mutation was called baroid (symbol B^{bd}).

2. In the generation in which baroid was discovered it behaved as a semi-dominant gene. In later generations it was practically completely recessive. The nature of the change in dominance relations is not known.

3. The behavior of baroid in heterozygotes with known bar allelomorphs is consistent with the view that baroid is an allelomorph of bar.

4. The locus of baroid is apparently identical with that of bar.

5. Baroid is associated with a reciprocal translocation involving an exchange of sections between the X and the second chromosome.

6. The X chromosome is broken at the locus of bar. There is no crossing over between baroid and the locus of breakage.

7. The second chromosome is broken at a locus lying close to the left of vestigial.

8. The results of the cytological investigation are in accord with the interpretation of the nature of the translocation given above.

9. The presence of the translocation decreases the frequency of crossing over in the X and in the right limb of the second chromosome, increases the frequency of non-disjunction of the X chromosomes, and decreases the effect of the inverted section C_{IIR} on crossing over in a certain region of the second chromosome.

10. The appearance of mutations from wild-type to bar can be accounted for by assuming that the effect of a gene on development may depend both upon its intrinsic properties and upon the properties of the neighboring genes. The assumption is derived from the position effect phenomenon discovered by STURTEVANT.

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APPENDIX

TABLE 8

$$\frac{y \quad c_v \quad v \quad f \quad 4 \quad +}{+ \quad 1 \quad + \quad 2 \quad + \quad 3 \quad + \quad B^{bd}} \; ♀ \times y \; c_v \; v \; f \; ♂.$$

MALES ONLY			
$0 - B^{bd}$	635	$1,2 - c_v \; B^{bd}$	9
$0 - y \; c_v \; v \; f$	726	$1,2 - y \; v \; f$	10
$1 - y \; B^{bd}$	104	$1,3 - c_v \; v \; B^{bd}$	8
$1 - c_v \; v \; f$	107	$1,3 - y \; f$	6
$2 - y \; c_v \; B^{bd}$	161	$2,3 - v \; B^{bd}$	7
$2 - v \; f$	168	$2,3 - y \; c_v \; f$	4
$3 - y \; c_v \; v \; B^{bd}$	81	$1,2,3 - c_v \; f$	1
$3 - f$	93		
$4 - y \; c_v \; v \; f \; B^{bd}$	—		
4 — wild-type	2	Total	2122

TABLE 9

$$\frac{+ \quad B^{bd} \quad +}{f \quad 1 \quad 2 \quad f_u} \; ♀ \times f \; f_u \; ♂.$$

MALES ONLY			
$0 - B^{bd}$	2466	$2 - B^{bd} \; f_u$	4
$0 - f \; f_u$	2094	$2 - f$	3
$1 - f_u$	1	Total	4568

TABLE 10

$$\frac{+ \quad B^{bd} \quad +}{f \quad 1 \; BB \quad 2 \quad f_u} \; ♀ \times f \; f_u \; ♂.$$

MALES ONLY			
$0 - B^{bd}$	5161	$2 - f \; BB$	11
$0 - f \; BB \; f_u$	3514	$2 - B^{bd} \; f_u$	3
$1 - f \; B^{bd}$	2	$0 - f \; f_u$ (non-disjunction)	486
$1? - 2? \; f \; B \; B^{bd}$	1	Total	9178

TABLE 11

$$\frac{+ \quad B^{bd} \quad +}{f \;\; 1 \;\; BB \;\; 2 \;\; f_u} \quad \frac{+ \qquad 3 \; +}{Cy \;\; C_{II\ L} \qquad L \;\; C_{II\ R}} \; ♀ \times f \, f_u \; ♂.$$

MALES ONLY			
$0 - B^{bd}$	1932	$3 - f \, BB \, f_u \, L$	23
$0 - f \, BB \, f_u \, C_y \, L$	1567	$0 - f \, f_u \, C_y \, L$ (non-disjunction)	23
$1 - BB \, f_u \, C_y \, L$	1	$3 - f \, f_u \, L$ (non-disjunction)	3
$2 - B^{bd} \, f_u$	3		
$2 - f \, BB \, C_y \, L$	3		
$3 - B^{bd} \, C_y$	49	Total	3604

TABLE 12

$$\frac{+ \quad B^{bd} \quad +}{g \;\; 1 \;\; + \;\; 2 \;\; c_r} \quad \frac{+ \qquad 3 \; +}{C_y \;\; C_{II\ L} \qquad L \;\; C_{II\ R}} \; ♀ \times g \, c_r \; ♂.$$

FEMALES		MALES	
$0 -$ wild-type	707	$0 - B^{bd}$	613
$0 - g \, c_r \, C_y \, L$	523	$0 - g \, c_r \, C_y \, L$	476
$1 - g$	40	$1 - g \, B^{bd}$	38
$1 - c_r \, C_y \, L$	31	$1 - c_r \, C_y \, L$	28
$2 - g \, C_y \, L$	2	$2 - B^{bd} \, c_r$	9
$2 - c_r$	2	$2 - g \, C_y \, L$	4
$3 - C_y$	34	$3 - B^{bd} \, C_y$	33
$3 - L \, g \, c_r$	30	$3 - g \, c_r \, L$	30
$1,3 - g \, C_y$	4	$1,3 - c_r \, L$	2
$1,3 - c_r \, L$	6		
Total	1379	Total	1233

TABLE 13

Crossing over in the second chromosome in the baroid stock.

$\frac{a_1\ 1\ d_p\ 2\ b\ 3\ p_r\ 4\ +\ 5\ c\ 6\ p_z\ 7\ s_p}{+\quad +\quad +\quad +\quad B^{bd}\quad +\quad +\quad +}$ ♀ × *f* $a_1\ d_p\ b\ p_r\ c\ p_z\ s_p$ ♂
(*forty-six males carrying f disregarded*).

MALES ONLY

$0 - B^{bd}$	180	$2,4 - b\ p_r\ B^{bd}$	11
$0 - a_1\ d_p\ b\ p_r\ c\ p_z\ s_p$	212	$2,4 - a_1\ d_p\ c\ p_z\ s_p$	8
$1 - a_1\ B^{bd}$	50	$2,5 - b\ p_r$	4
$1 - d_p\ b\ p_r\ c\ p_z\ s_p$	36	$2,6 - b\ p_r\ c$	45
$2 - a_1\ d_p\ B^{bd}$	96	$2,6 - a_1\ d_p\ B^{bd}\ p_z\ s_p$	33
$2 - b\ p_r\ c\ p_z\ s_p$	126	$2,7 - b\ p_r\ c\ p_z$	6
$3 - a_1\ d_p\ b\ B^{bd}$	22	$2,7 - a_1\ d_p\ B^{bd}\ s_p$	6
$3 - p_r\ c\ p_z\ s_p$	30	$3,4 - p_r\ B^{bd}$	2
$4 - a_1\ d_p\ b\ p_r\ B^{bd}$	25	$3,4 - a_1\ d_p\ b\ c\ p_z\ s_p$	3
$4 - c\ p_z\ s_p$	12	$3,5 - p_r$	2
$5 - a_1\ d_p\ b\ p_r$	4	$3,6 - p_r\ c$	8
$5 - B^{bd}\ c\ p_z\ s_p$	2	$3,6 - a_1\ d_p\ b\ B^{bd}\ p_z\ s_p$	4
$6 - a_1\ d_p\ b\ p_r\ c$	59	$3,7 - p_r\ c\ p_z$	3
$6 - B^{bd}\ p_z\ s_p$	65	$4,6 - c$	1
$7 - a_1\ d_p\ b\ p_r\ c\ p_z$	11	$5,6 - a_1\ d_p\ b\ p_r\ p_z\ s_p$	3
$7 - B^{bd}\ s_p$	23	$6,7 - a_1\ d_p\ b\ p_r\ c\ s_p$	2
$1,2 - d_p\ B^{bd}$	6	$2,3,7 - b\ B^{bd}\ s_p$	1
$1,2 - a_1\ b\ p_r\ c\ p_z\ s_p$	2	$2,4,6 - b\ p_r\ B^{bd}\ p_z\ s_p$	3
$1,3 - d_p\ b\ B^{bd}$	7	$2,4,6 - a_1\ d_p\ c$	2
$1,3 - a_1\ p_r\ c\ p_z\ s_p$	9	$2,3,6 - a_1\ d_p\ p_r\ c$	2
$1,4 - d_p\ b\ p_r\ B^{bd}$	1	$1,2,4 - a_1\ b\ p_r\ B^{bd}$	1
$1,4 - a_1\ c\ p_z\ s_p$	5	$1,3,6 - a_1\ p_r\ c$	2
$1,5 - d_p\ b\ p_r$	4	$1,6,7 - d_p\ b\ p_r\ c\ s_p$	2
$1,6 - a_1\ B^{bd}\ p_z\ s_p$	12	$2,6,7 - b\ p_r\ c\ s_p$	1
$1,6 - d_p\ b\ p_r\ c$	18	$2,4,7 - b\ p_r\ B^{bd}\ s_p$	2
$1,7 - a_1\ B^{bd}\ s_p$	5	$1,2,4,6 - a_1\ b\ p_r\ B^{bd}\ p_z\ s_p$	1
$1,7 - d_p\ b\ p_r\ c\ p_z$	1		
$2,3 - b\ B^{bd}$	3		
$2,3 - a_1\ d_p\ p_r\ c\ p_z\ s_p$	9	Total	1193

THE LINKAGE RELATIONS OF CERTAIN GENES IN OENOTHERA

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One may now predict with some confidence that two characters, which are known to segregate independently in certain Oenothera crosses, will be found to be closely linked in other crosses. Instances of this sort are increasingly numerous. RENNER (1925, 1928) was the first to call proper attention to such differences in linkage relationships, though other cases of the same sort had been discussed earlier by DE VRIES, by RENNER, and by BLANCHARD and BARTLETT.

At present there are only two characters that do not show such variable linkage relationships. The better known case involves the flower size differences found in many species (C_o, etc. of RENNER 1925). The second is the *brevistylis* character (short styles and deformed stigmas—b_r of SHULL [1926]) of *Oe. Lamarckiana*. The two characters are alike in that they have never been found to be linked to any other characters, even when all other characters in a hybrid were linked through the presence of all 14 chromosomes in a single ring.

VARIABLE LINKAGE RELATIONSHIPS

A difference in the linkage relationships of *nanella* stature (*n*) was reported by DE VRIES (1913, p. 235) in the cross *Oe.* "*biennis*-Chicago" (that is, *Oe. chicagoensis*) × *Oe. Lamarckiana nanella* which produced twin hybrids in F_1. The *densa* twin (that is, *excellens·gaudens*, probably with the

configuration 10, 2, 2[1], with chromosomes 1·2 and 5·6 as pairs, and with *N* in 3·4 of *excellens* and therefore in the ring [EMERSON and STURTEVANT 1931]) bred true for tall stature and other F_1 characteristics; in other words *n* was completely linked to the *gaudens* lethal (*gaudens* lethal not in 1·2 or 5·6 and therefore in the ring) and to other characters by which these complexes differed. In the *laxa* twin (that is, *excellens·n velans*, probably with the configuration 6, 2, 2, 2, 2, with chromosomes 1·2, 3·4, 11·12, and 13·14 as pairs, and with *n* in the pairing chromosome 3·4) *nanella* appeared in 35 percent of the progeny; in other words *n* was independent of the *velans* lethal. In the *Oe. Lamarckiana* parental race (*velans·gaudens*), the gene *n* and the *velans* and *gaudens* lethals were almost completely linked (DE VRIES 1913, many examples).

RENNER (1917, pp. 221–225) reported that red mid-ribs (*R*) segregated independently of other genes in *rubens·velans* (probable configuration 12, 2, with *R* in the pairing chromosome 1·2 [EMERSON and STURTEVANT 1931]), while in *albicans·R gaudens* (probable configuration 8, 6) *R* was completely linked to the other genes involved (the genes in *rubens* and in *gaudens* were identical as far as could be determined).

The case reported by DOCTOR (COBB) BLANCHARD (COBB and BARTLETT 1919, COBB 1921, BLANCHARD 1929) was in *Oe. pratincola*, in which the β complex of strain E carried a recessive gene (*f*) for revolute leaves which was masked by the dominant in the α complex. *Oe. pratincola* E ($\alpha\cdot\beta$) had a ring of 14 chromosomes (KULKARNI 1929) and was heterozygous for several characters all showing complete linkage except that *f* became associated with α in about one percent of the gametes. When the new *f*-α was crossed to the β (*F*) of *Oe. pratincola* strain C, the F_1 had the configuration 12, 2 (KULKARNI 1929) and *f* was inherited independently of all other characters of α and β.

In his 1928 paper, RENNER gave a summary of the linkage relationships observed by him in different hybrids. For example, in *curvans·flavens* S_p[2] was linked to C_u, and *M* and *B* were independent, while in *flavens·flectens* S_p was linked to *B* with C_u and *M* independent. In *flavens·velans* (probable configuration 4, 4, 2, 2, 2) S_p and *B* were linked and *P*(*M*) and *R* were linked but independent of the former group. In *rubens·velans* (probable configuration 12, 2) *P*, *B* and S_p were linked and *R* was independent,

[1] Chromosome configurations are indicated by the convention used by STURTEVANT (1931). The numbers separated by commas indicate the numbers of chromosomes in the rings (or pairs): 10, 2, 2 indicates a ring of 10 and 2 pairs; 8, 6 indicates a ring of 8 and a ring of 6, etc.

[2] S_p is a gene for pointed buds and leaves, C_u for curved stems, *M* for marginate leaves (allelomorph of *P*, a gene for punctate stems), *B* for broad leaves, and *R* for red mid-ribs.

while in *curvans·rubens* and *curvans·velans* *R*, *P*(*M*), *B*, S_p and C_u were all linked. In all the cases reported by RENNER the linkage was either extremely close or entirely lacking.

A similar situation was found by SHULL (1923a) in crosses between *Oe. Lamarckiana* and *Oe. rubricalyx* in which P^r (*rubricalyx* bud color) was linked to the *velans* and *gaudens* lethals in *gaudens*·modified-*velans* with the configuration 12, 2 (the pair probably was chromosome 1·2), but P^r was independent of the *velans* lethal in *ʰlatifrons·velans* with the configuration 8, 2, 2, 2 in which P^r was in the pairing chromosome 3·4 (cytology and interpretation by EMERSON [1930] and EMERSON and STURTEVANT [1931]). Linkage of sulfur flower color (*s*) and dwarf stature (*d*) to striped bud color (P^s) and to the *sulfurens* pollen lethal was found in *ʰfranciscana·sulfurens* with the configuration 10, 2, 2, while *s* and *d* were completely independent in *ʰfranciscana·ʰfranciscana* with 7 paired chromosomes (EMERSON 1931a). SHULL (1926) has found *n* (*nanella* stature) and *v* (old-gold flower color) to be independently inherited in all his crosses, but STURTEVANT (1931) found them to be very closely linked in *jugens·N* which had the configuration 10, 2, 2, with *v* in chromosome 1·2 and *n* in chromosome 3·4, both in the ring. There are many other cases of the same sort (see especially GERHARD 1929, and CLELAND and OEHLKERS 1930).

The apparent rule is that characters which are independently inherited in hybrids with pairing chromosomes will be linked in hybrids in which the chromosomes are in rings. By means of this rule it has been possible to identify certain genes with particular chromosomes (EMERSON and STURTEVANT 1931, pp. 410–412), as has been noted in some of the crosses reviewed above.

LINKAGE WITHIN CHROMOSOME 1·2

Chromosome 1·2 is defined as that chromosome which is present in *ʰHookeri* and in *gaudens* but not in *flavens* (EMERSON and STURTEVANT 1931, p. 403) and corresponds to RENNER's linkage group II and to SHULL's III. The gene *R* (red mid-ribs) is in chromosome 1·2 since it is independent of certain other characters in plants in which 1·2 is a pairing chromosome, but is linked to the same characters in plants in which 1·2 is in the ring (EMERSON and STURTEVANT 1931, p. 410).

Old-gold flower color has recently been found to be in chromosome 1·2. The evidence for placing *v* (old-gold) in chromosome 1·2 is as follows: The F_1 hybrid *R velans·N* had the configuration 6, 2, 2, 2, 2 and was heterozygous for the factor pairs *R r*, *V v*, *N n*, and P^r P^s (STURTEVANT 1931).

The backcross to N ($r\ v\ n\ P^r$), using the multiple recessive as pollen parent, gave the following frequencies (culture 18 of 1931):

5 *R V N* P^r
1 *R V n* P^r
5 *r v N* P^r
4 *r v n* P^r

In the backcross of *R velans*·*N* to an *r v N* P^s, extracted from *franciscana*·*N* crosses, the following frequencies were observed (culture 20 of 1931):

9 *R V N* P^r
1 *r V N* P^r
7 *r v N* P^r
1 *r v N* P^s[3]

The F_1 hybrid *R gaudens*·*N* had the configuration 10, 2, 2 and was heterozygous for the factor pairs *R r*, *V v*, and P^r *p* (STURTEVANT 1931). In the backcross to the extracted *r v* P^s (culture 19 of 1931) there were 2 *R V* P^r and 1 *r v* P^r.

In *Oe. R Lamarckiana* the gene *R* is carried in the pairing chromosome 1·2 which is common to *velans* and *gaudens*. Chromosome 1·2 is also carried in the complex *N* (EMERSON and STURTEVANT 1931). The pair 1·2 in *R velans*·*N* and in *R gaudens*·*N* is therefore identical and the linkage data for this chromosome should be comparable in both hybrids. In the backcrosses reported above there were 35 non-crossover gametes recovered (17 *R V* and 18 *r v*) and one crossover gamete (*r V*). This represents about 3 percent crossing over between *R* and *v*, but the data are too few to give an accurate indication of the linkage intensity. Since 1·2 was known to be a pairing chromosome in these hybrids, and since *R* is known to be in 1·2, it follows that *v* is also in 1·2. It should be noted in the *R velans*·*N* backcross that *n* segregated independently of the characters in chromosome 1·2.

It is probable that b_u (*bullata*) and s_p (double flowers), which SHULL (1925, 1927, 1928) has found to be linked to *v*, are also in chromosome 1·2 since most of his crosses were in *Oe. Lamarckiana* in which 1·2 is a pair.

LINKAGE WITHIN CHROMOSOME 3·4

Chromosome 3·4 is defined as that chromosome which is present in *Hookeri* but not in *gaudens* and not in *flavens* (EMERSON and STURTEVANT

[3] This plant and the *P* recorded below from *flavens*·*N* selfed suggest mutation of P^r to *P*.

1931, p. 403) and corresponds to SHULL's linkage group I and to RENNER's I. The gene *P* (punctate stems) is in chromosome 3·4 since it is inherited independently of *R* in any hybrids in which 3·4 is a pairing chromosome but is linked to *R* in all hybrids in which both 1·2 and 3·4 are in a common ring (EMERSON and STURTEVANT 1931, p. 410). There are probably a series of allelomorphs of *P* (*P*^{r}, *rubricalyx* bud color with punctate stems; *P*^{s}, striped bud cones with punctate stems; *P*, green buds with punctate stems; and *p*, green buds with no punctation on the stems [see discussion in EMERSON 1931b, pp. 390–392]).

SHULL (1923b) found linkage with about 8 percent crossing over between *P*^{r} *P*^{s} and *S s* (yellow *versus* sulfur flowers) in certain crosses in which it was not known that 3·4 was a pairing chromosome. Since then EMERSON (1931b) has found the same relationship in the hybrid *s d* ^{h}*franciscana*·^{h}*latifrons* (configuration 4, 2, 2, 2, 2, 2) in which 3·4 was known to be a pairing chromosome (EMERSON and STURTEVANT 1931) and in which it was shown by cytological examination of plants in the backcross to *s d* ^{h}*franciscana* that both *P* and *s* were independent of the only ring present in the hybrid.

SHULL also found *n* to be linked to *s* and to *P*^{r}, but here again it was not known that 3·4 was a pairing chromosome. STURTEVANT (1931) has since found *P*^{r} and *n* to be linked in the hybrid ^{h}*franciscana*·*N* (configuration 6, 2, 2, 2, 2) in which chromosome 3·4 was known to be one of the pairs, and EMERSON (1931b) has found *n* and *s* to be linked in the hybrid *s d* ^{h}*franciscana*·*n velans* (configuration 6, 2, 2, 2, 2) in which 3·4 was again known to be one of the pairs.

From the above data it is evident that *P*, *n*, and *s* are all in chromosome 3·4 and that the observed linkage between these genes is due to their presence in a single chromosome. There is now additional data to support this conclusion. In the hybrid *s d* ^{h}*franciscana*·*N* (configuration 6, 2, 2, 2, 2[EMERSON 1931b]) chromosome 3·4 was known to be one of the pairs (EMERSON and STURTEVANT 1931). The following frequencies were observed in F_2 (culture 2305 of 1931):

14 *P*^{r} *S N V D*
5 *P*^{r} *S N V d*
3 *P*^{r} *S N v D*
2 *P*^{r} *S n V D*
1 *P*^{r} *S n V d*
2 *P*^{r} *S n v d*
1 *P*^{s} *S N v D*
3 *P*^{s} *s N V D*

The genes *v* and *d* (dwarf stature) were independent of the genes in chromosome 3·4. Disregarding these two genes, the frequencies for the genes in 3·4 were: 22 *P*^{r} *S N*, 5 *P*^{r} *S n*, 1 *P*^{s} *S N*, and 3 *P*^{s} *s N*, indicating very close linkage between these three genes. Unfortunately, there was no triple recessive to use for backcrossing, but backcrosses were obtained for *P*^{s} *n* (4 percent crossing over, total equals 25) and for *P*^{s} *s* (12.5 percent crossing over, total equals 40).

Revolute leaves (*f*) have been supposed by SHULL (1923b) to be in the same linkage group as *P*, *s*, and *n*. We have found close linkage with no crossing over between *f* and *s* in extensive cultures, but in no case has it been known that these genes were in pairing chromosomes.

The *velans* zygotic lethal which was similarly supposed to be in the same linkage group (SHULL 1923a, 1923b) has since been shown (EMERSON 1930) to be in a different chromosome. In the hybrid *^{h}latifrons·velans*, the configuration was 8, 2, 2, 2 and the factor pair *P*^{r} *P*^{s} was shown to be in one of the pairing chromosomes while the *velans* zygotic lethal was in the ring of 8 (since the only segregates with 7 pairs of chromosomes were *^{h}latifrons·^{h}latifrons*).

LINKAGE BETWEEN CHROMOSOMES 1·2 AND 3·4

Linkage between *R* in chromosome 1·2 and *P* in 3·4 was found by RENNER (1925) in *velans·flavens*. In this hybrid it is known that 1·2 and 3·4 of *velans* are in a ring of 4 with 1·4 and 2·3 of *flavens* (EMERSON and STURTEVANT 1931).

Linkage between *v* in chromosome 1·2 and *n* in 3·4 was found by STURTEVANT (1931) in the hybrid *jugens· N*. This hybrid had the configuration 10, 2, 2 and the pairs were 8·13, 11·12, chromosomes 1·2 and 3·4 of *N* being in the ring of 10 (EMERSON and STURTEVANT 1931). In addition to the counts previously recorded there was an F_2 with 8 *V P*^{r} *N* and 1 *v P*^{r} *n* (culture 25 of 1931).

The hybrid *flavens·N* had the configuration 4, 4, 2, 2, 2 (STURTEVANT 1931). One of the rings of 4 was made up of chromosomes 1·2 and 3·4 of *N* with 1·4 and 2·3 of *flavens*. The F_2 of this cross (culture 23 of 1931) showed linkage between *v*, *P*^{r} and *n*; the frequencies were 5 *p N V* (*lutescens*), 6 *P*^{r} *N V*, 1 *P*^{r} *n v*, and 1 *P N V*. The backcross to *N* (*P*^{r} *n v*) showed complete linkage between *n* and *v*—6 *P*^{r} *N V* to 6 *P*^{r} *n v* (culture 22 of 1931).

In the hybrid *accelerans·N*, *v* was found to be linked to *P*^{r}. The F_1 had the configuration 12, 2 (STURTEVANT 1931) with the pair known to be 5·6 (EMERSON and STURTEVANT 1931). In the backcross to *v P*^{s} *N* the following frequencies were obtained (culture 36 of 1931): 12 *v P*^{r} with erect

mid-rib hairs and appressed sepal tips (that is, the *N* complex was recovered) and 2 *V* P^s with strigose mid-rib hairs and spreading sepal tips (that is, the *accelerans* complex was recovered).

The F_1 *sulfurens·v ʰfranciscana* had the configuration 10, 2, 2, and was heterozygous for the factor pairs P^s *p*, *S s*, *V v*, The pairs were known to be 7·10 and 8·9 (EMERSON and STURTEVANT 1931). In the backcross to P^s *v s* there were (cultures 2380, 2381 of 1931): 17 *v*, P^s P^s, *S*; 4 *V*, P^s *p*, *s*; and 1 *V*, P^s *p*, *S*. The last plant was examined cytologically and found to have the configuration 10, 2, 2, indicating the constitution *S sulfurens·s v ʰfranciscana*. Otherwise there were no crossovers between *v* in chromosome 1·2 and P^s and *s* in chromosome 3·4.

LINKAGE RELATIONS OF *d* (DWARF STATURE)

It has previously been shown (EMERSON 1931a) that dwarf stature (*d*) was independent of *s* (chromosome 3·4) in a hybrid with 7 pairs of chromosomes, and that *d* was independent of both *s* and P^r in another hybrid with the configuration 4, 2, 2, 2, 2, 2 in which chromosome 3·4 was known to be a pair (*s d ʰfranciscana·ʰlatifrons* [EMERSON 1931b]). It has also been shown that *d* was in the ring of 10 in *sulfurens·s d ʰfranciscana* and consequently independent of the pairing chromosomes 7·10 and 8·9 (EMERSON 1931a; for chromosome identities see EMERSON and STURTEVANT 1931). In the F_2 from *s d ʰfranciscana·N* (described in the third section of this paper), *d* was independent of P^r, *s*, and *n* in chromosome 3·4 and also independent of *v* in chromosome 1·2.

The F_1 *s d ʰfranciscana·b_r velans* had the configuration 6, 2, 2, 2, 2 (EMERSON 1931b). In the backcross to *s d ʰfranciscana* there were (culture 2293 of 1931): 4 *S D* (2 plants with 7 pairs of chromosomes), 3 *S d* (1 with 7 pairs, 1 with configuration 6, 2, 2, 2, 2,), 1 *s D*, and 3 *s d* (1 with 7 pairs, 1 with 6, 2, 2, 2, 2). In the plants examined cytologically, *s* and *d* were each associated with the ring chromosomes in their inheritance in 2 cases and not so associated in 4 cases, indicating that neither *s* nor *d* was in the ring of 6. The ring in this hybrid was made up of chromosomes 5·8, 6·7 and 9·10 of *velans* and 5·6, 7·10 and 8·9 of *ʰfranciscana*.

The F_1 *jugens·s d ʰfranciscana* had the configuration 10, 2, 2 (STURTEVANT 1931) with chromosomes 7·10 and 11·12 as pairs (EMERSON and STURTEVANT 1931). In the F_2 (culture 26 of 1931) there were 12 *D S* and 3 *d s*. In the backcross *s d ʰfranciscana* × F_1 (culture 28 of 1931) there were 2 *D S* and 13 *d s*. Chromosome 3·4, which carried *s*, was in the ring of 10 in the F_1, and, since *s* and *d* were linked, *d* must also have been in the ring of 10 and consequently independent of chromosomes 7·10 and 11·12.

In the crosses reported above, *d* (dwarf stature) has been shown to be inherited independently of chromosomes 1·2, 3·4, 5·6, 7·10, 8·9 and 11·12 of *hfranciscana*, and consequently may be supposed to be carried in chromosome 13·14. A direct test of this point was available in the backcross of *s d hfranciscana·N* to *s d hfranciscana* in which *d* should never have been associated with the ring of 6 (chromosomes 7·10, 8·9 and 13·14 of *hfranciscana*), but, unfortunately, the proper plants were not examined cytologically.

FLOWER SIZE AT THE C_o LOCUS[4]

The best evidence of the independence of flower size was found by LANGENDORF (1930) in crosses between *Oe. biennis* and *Oe. Hookeri*. The F_1 *albicans·hHookeri* has been found usually to have all 14 chromosomes in a single ring (CLELAND and BLAKESLEE 1930). Due to the presence of a pollen lethal in *balbicans*, all functioning pollen was *hHookeri*, and through the great superiority of *hHookeri* over *balbicans* in gametophytic competition (RENNER 1921), all functioning eggs were also *hHookeri*. The F_2 thus consisted entirely of homozygous *Hookeri* plants and these were uniform for all characters involved except flower size. Of the parents, *Oe. biennis* had the smaller flowers (petal length 13 to 19 mm) and *Oe. Hookeri* had much larger (34 to 40 mm). The flower size of the F_1 was definitely intermediate (23 to 31 mm). In the F_2, flower size segregated sharply into two classes. Tests in the F_3 generation and backcrosses to *Oe. Hookeri* showed that small flower size was a simple Mendelian dominant in plants of the constitution *hHookeri·hHookeri*. LANGENDORF'S data for petal length in these crosses are summarized in table 1.

From this hybrid, *hHookeri* chromosomes alone were recovered in the F_2 and backcrosses; still half of the gametes carried the gene C_o (small flower size) from *balbicans*. Thus C_o is shown to have been inherited independently of all the chromosomes of *balbicans*. It has been suggested (EMERSON and STURTEVANT 1931, p. 414) that these conditions can be satisfied by the assumption that the gene had its locus at such a distance from the translocation point in some chromosome as to give 50 percent crossing over with that point.

[4] In comparing the measurements of flower size in different crosses and in the data of different authors, it should be borne in mind that the measurements reported cannot be exact. Flower size on a given plant may vary somewhat during the flowering season (see GATES 1917). A more important error can arise from measuring flowers at different times of day since the petals grow considerably between the time of opening in the evening and the time that the flowers wilt the following morning. Differences in petal shape in various species also make exact measurements difficult.

The case for *albicans·velans* (HOEPPENER and RENNER 1929) is not quite as clear as in the cross just described, since the data were not given in detail, but has one advantage in that cytological examinations were made on the plants studied. HOEPPENER and RENNER found that both large and small flowered F_2 plants had rings of 14 chromosomes.

CLELAND and OEHLKERS (1930) reported independent segregation for flower size in two hybrids, each having a ring of 14 chromosomes. The F_2 from *Hookeri·truncans* consisted of 27 plants of the F_1 constitution of which 17 had small flowers and 10 had large flowers, and 17 homozygous *Hookeri* plants of which 9 had small flowers and 8 large. The F_2 from *acuens·gaudens* bred true except for flower size: there were 38 plants with small flowers and 23 with large.

RUDLOFF (1921) reports that the small flowers of *Oe. purpurata* were dominant to the larger ones of *Oe. Hookeri* (F_1 could have not more than two pairs)[5] and that there was a simple monohybrid segregation, tested by raising F_2 and both types of backcrosses from both F_1's (*Hookeri* × *purpurata* and *purpurata* × *Hookeri*). There was also recombination for flower-size (not clearly monofactorial) in *purpurata·velans* (12, 2), *purpurata·gaudens* (10, 4 or 10, 2, 2), and *purpurata·albicans*. In these cases there can be no single chromosome of *purpurata* that is segregating freely of the rest in all cases, since *albicans* has no chromosome in common with *Hookeri*, *velans*, or *gaudens* (EMERSON and STURTEVANT 1931).

Students of Oenothera have accumulated numerous instances of the type of inheritance of flower size reported in the crosses reviewed above. Only a partial list of such cases will be presented.

Among the cases reported by DE VRIES is the cross *Oe. muricata* × *Oe. biennis* in which the F_1 (that is, *rigens·rubens*) was intermediate between the parents for flower size (*Oe. muricata* had smaller flowers than *Oe. biennis*). The F_2 bred true for all characters except flower size which varied from "fast *muricata*-Grösse bis *biennis* ab. Diese Fluktuation (war) eine individuelle, die eine Pflanze (war) grossblumig an allen Tagen und auf allen Zweigen, die andere aber stets kleinblumig oder mittelblumig" (DE VRIES 1913, pp. 41–43). Similar results were obtained in the cross *Oe. muricata* × *Oe. Lamarckiana*, F_1 *velutina* (that is, *rigens·velans*) in which the F_2 segregated into two types "deren einer doppelt so grosse Blüten

[5] RUDLOFF's cytological results are based on F_2 and backcross individuals. An extracted *purpurata·velans* had 12, 2, which must also have been the configuration of the F_1. An extracted *purpurata·gaudens* had 10, 2, 2, which means that the F_1 was either 10, 2, 2 or 10, 4. The genetic results suggest that *purpurata* carries chromosome 5–6; if this deduction is correct *purpurata·gaudens* is 10, 2, 2; and *purpurata·Hookeri* is also 10, 2, 2.

hat als der andere" (DE VRIES 1913, pp. 127–128). Similar conditions were found in other crosses.

Among the cases reported by DAVIS are the intercrosses between *Oe. franciscana* and *Oe. biennis*. Petal length in *Oe. franciscana* was 30 to 35

TABLE 1

Petal length (in mm) in crosses between Oe. biennis and Oe. Hookeri, data summarized from LANGENDORF *(1930) tables 1–28.*

TABLE	CROSS	10	11	12	13	14	15	16	17	18	19	20	21
1	*Oe. biennis*	..	..	..	1	2	6	4	7	5	2	..	..
	Oe. Hookeri	..	..	..	..	..	..	..	..	..	..	..	..
2	*biennis*×*Hookeri*	..	..	..	..	..	..	..	..	..	..	..	..
4	(B×H)F_2	..	1	8	12	17	18	25	41	17	6	2	1
5	F_1×*Hookeri*	..	1	2	..	5	10	15	7	6	9	3	2
6	*Hookeri*×F_1	..	..	..	..	1	2	2	5	3	2	1	..
7	F_2 large×self	..	..	..	..	..	..	..	..	..	..	..	..
8	F_2 large×self	..	..	..	..	..	..	..	..	..	..	..	..
9	F_2 large×self	..	..	..	..	..	..	..	..	..	..	..	..
10	F_2 small×self	..	..	..	..	3	1	5	4	5	4	..	..
11	F_2 small×self	..	..	..	..	..	..	2	..	9	14	4	..
12	F_2 small×self	..	3	4	9	10	15	11	7	1	..	..	..
13	F_2 small×*Hookeri*	..	..	..	..	..	1	3	12	11	6	2	..
14	(F_1×H) large×self	..	..	..	..	..	..	..	..	..	..	..	..
15	(F_1×H) large×self	..	..	..	..	..	..	..	..	..	..	..	..
16	(F_1×H) large×self	..	..	..	..	..	..	..	..	..	..	..	..
17	(F_1×H) small×self	1	3	2	4	3	5	8	6	5	1	..	..
18	(F_1×H) small×*Hookeri*	..	..	..	..	..	1	3	12	11	6	2	..
19	F_2 large×*flavens*	..	..	..	..	..	..	..	..	..	..	..	..
20	F_2 large×*flavens*	..	..	..	..	..	..	..	..	..	..	..	..
21	F_2 small×*flavens*	..	..	..	..	..	..	..	..	..	..	..	..
22	F_2 small×*flavens*	..	..	..	..	..	..	..	..	..	..	..	..
23	*flavens*×F_2 large	..	..	..	..	..	..	..	..	..	..	..	..
	albicans×F_2 large	..	..	..	..	..	..	..	..	..	..	..	..
24	*flavens*×F_2 small	..	..	..	..	..	..	..	..	..	..	..	..
	albicans×F_2 small	..	..	..	..	..	..	..	..	..	..	..	..
25	*flavens*×F_2 small	..	..	..	..	..	..	..	..	..	..	..	..
	albicans×F_2 small	..	..	..	..	..	..	..	..	..	..	..	..
26	F_1×*biennis*	..	..	..	1	..	..	..	7	16	15	17	2
27	(F_1×H) large×*biennis*	..	..	..	..	..	..	2	..	5	14	18	11
28	(F_1×H) small×*biennis*	..	..	1	..	..	2	3	3	3	10	14	3

mm and in *Oe. biennis* 20 to 23 mm. The *franciscana*×*biennis* F_1 (that is, *franciscana·rubens*, probably with the configuration 10, 2, 2) had petals 21 to 24 mm long. The F_2 consisted of 64 plants of the F_1 constitution of which 7 had large flowers, 15 intermediate and 42 small; and 219 *francis-*

cana-like plants (that is, ^{h}*franciscana*·^{h}*franciscana*) of which 54 were large flowered, 38 intermediate and 127 small; the small flowered group in both types contained plants with smaller flowers than the *biennis* parent. The reciprocal F_1 (that is, ^{b}*albicans*·^{h}*franciscana*, with the probable configuration either 14, or 12, 2) had petals 23 to 25 mm in length and gave in F_2 20 plants of the F_1 type, of which 3 had large flowers, 5

TABLE 1 (continued)

22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	TABLE
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
..	..	..	..	..	..	..	..	..	..	..	..	1	3	5	8	8	4	1	..	1
..	1	2	4	7	8	6	4	2	1	..	..	..	..	..	..	..	..	..	..	2
2	..	2	4	6	5	10	12	10	6	9	18	4	3	1	2	..	..	..	..	4
..	2	1	1	3	5	6	8	6	8	9	8	10	3	3	3	1	..	..	..	5
1	1	..	..	..	..	2	3	8	7	5	7	7	4	1	2	2	..	..	..	6
..	..	..	..	..	..	..	..	..	1	..	9	11	15	15	3	5	2	..	..	7
..	..	..	..	..	..	..	2	1	4	7	9	10	7	1	3	..	..	..	..	8
..	..	..	..	1	3	2	..	15	19	18	9	5	6	..	..	..	..	..	..	9
..	1	..	..	..	..	..	1	1	2	3	..	..	2	2	1	..	..	..	..	10
..	..	..	..	..	..	..	..	..	..	1	2	1	1	..	..	..	..	..	..	11
..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	12
..	..	..	..	..	..	..	1	2	3	7	8	3	6	2	..	1	..	..	..	13
..	..	..	..	..	..	..	2	4	9	8	8	10	7	3	1	1	..	..	..	14
..	..	..	..	..	..	1	2	6	11	24	52	38	39	18	8	5	1	..	1	15
..	..	..	..	..	..	1	..	..	2	7	6	19	17	8	7	..	3	..	..	16
..	..	..	2	..	..	2	1	2	1	3	2	..	..	1	..	..	..	..	..	17
..	..	..	..	..	1	..	..	..	2	3	8	6	3	3	1	..	..	..	..	18
..	..	2	13	11	10	8	10	4	2	..	1	..	..	..	..	..	..	..	..	19
..	..	..	1	1	..	2	6	12	18	7	1	1	..	..	..	..	1	..	..	20
..	1	..	10	21	18	8	2	..	..	1	1	..	..	..	..	..	..	..	..	21
1	..	5	8	8	14	9	5	2	4	..	..	..	..	..	..	..	..	..	..	22
..	2	2	5	12	18	18	21	20	9	3	1	..	..	..	..	..	..	..	..	23
..	..	1	..	6	6	3	2	1	1	..	..	..	..	..	..	..	..	..	..	
..	2	1	11	14	13	10	10	3	..	..	..	..	..	..	..	..	..	..	..	24
..	..	2	6	6	1	4	..	1	3	..	..	..	..	..	..	..	..	..	..	
..	..	..	4	7	3	6	10	6	1	2	..	..	..	..	..	..	..	..	..	25
1	1	2	4	4	6	4	..	3	2	..	..	..	..	..	..	..	..	..	..	
4	3	2	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	26
1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	27
1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	28

intermediate and 12 small, and 96 *franciscana*-like plants of which 6 had large flowers, 28 intermediate and 62 small (DAVIS 1914, pp. 175, 189, 191; 1916, pp. 207, 215, 221–227). In the double reciprocal cross (*Oe. biennis*×*Oe. grandiflora*)×(*Oe. grandiflora*×*Oe. biennis*), there was segre-

gation for other characters as well as flower size, but "no correlations were discovered between flower size and any other features of morphology" (DAVIS 1917, p. 179).

GATES (1917) reported that flower size was intermediate between that of the parents in reciprocal F_1's in crosses between *Oe. rubricalyx* and *Oe.* "*biennis*" (a form collected from the wild in England), and that in the F_2 generations flower size was extremely variable. His observations on "somatic segregation" within individual plants are not germane to the present discussion since the variants were not tested genetically.

Among the cases reported by RENNER is the backcross *[b]albicans·curvans* × *rubens* (*[b]albicans·rubens* = *Oe. biennis*, petal length 28 mm; *curvans* from *Oe. muricata*, petal length 15 mm; F_1 *[b]albicans·curvans* had petal length 18 mm, probable configuration 6, 4, 2, 2) in which part of the plants had the flower size of *biennis*, the other being smaller (RENNER 1917, pp. 159–161, 167, 203). In the cross *Oe. biennis* × *Oe. Lamarckiana*, the three F_1 types (*[b]albicans·gaudens*, *[b]albicans·velans*, *rubens·velans*) had petals of about 28 mm in length (*Lamarckiana* had petals about 48 and *biennis* about 28 mm in length). The F_2 from the *rubens* F_1 segregated into three types for flower size (about 50, 40, and 30 mm). The backcross to *biennis* gave only small flowered plants (except for some difference between *velans* and *rubens* twins) while the backcross to *Lamarckiana* segregated for flower size much in the manner of the F_2 (RENNER 1917, pp. 159–161, 178, 197–199, 204–207). In *Oe. Hookeri* × *Oe. Cockerelli* F_1 *[h]Hookeri·elongans*, the petal length was 22 mm, and in the F_2 petal length varied from 21 to 40 mm. In the backcross *Oe. Hookeri* × *curtans·[h]Hookeri*, petal length varied from 23 to 43 mm (20 mm in the F_1) (RENNER 1925).

There is apparently a single locus responsible for such differences in flower size. RENNER (1925, p. 132) treats the various differences in flower size as due to a series of allelomorphs, C_o, C_{o1}, c_o, etc. The probable truth of this conclusion is evident when one compares the inheritance of flower size in such hybrids as *rigens·rubens*, *rigens·velans* and *[h]franciscana·rubens* in which three different flower sizes (small in *rigens*, intermediate in *rubens*, and large in *velans* and *[h]franciscana*) follow the same type of inheritance.

OTHER LOCI AFFECTING FLOWER SIZE

Not all inherited differences in flower size follow the scheme of inheritance described for C_o. In his description of the genes for flower size belonging to the C_o series of multiple allelomorphs, RENNER (1925, p. 132) states: "Dass die Blütengrösse auch durch andere Faktoren beeinflusst

wird, versteht sich von selbst. Als solche sind bekannt: $B>b$, $R>r$, p von *flavens* $>P$ von *velans*."

An indication of the influence of the p carrying chromosome of *flavens* on the expression of flower size was obtained in crosses between *Oe. "franciscana sulfurea"* (*sulfurens·s d ʰfranciscana*) and *Oe. suaveolens* (*albicans·flavens*). The F_1 *s d ʰfranciscana·flavens* had the configuration 4, 4, 2, 2, 2 (EMERSON 1931b). The rings of 4 involved chromosomes 1·2, 3·4 of *ʰfranciscana* with 1·4, 2·3 of *flavens*, and 7·10, 8·9 of *ʰfranciscana* with 7·8, 9·10 of *flavens*. The second ring was unmarked genetically, but the first could be followed by the genes *s* (sulfur flower color) and P^s (punctate stems, striped bud cones) in chromosome 3·4 of *ʰfranciscana* (EMERSON and STURTEVANT 1931). There was no great difference in flower size between *"franciscana sulfurea,"* homozygous *s d franciscana*, *Oe. suaveolens* and the various F_1 hybrids (petal length about 35 to 40 mm in each). The F_2 from *s d ʰfranciscana·flavens* (culture 2286 of 1931) gave the following frequencies:

24 P^s *S* large (22*D*, 2*d*)	petal length 35 to 40 mm
4 *p S* large (4*D*, 0*d*)	
3 P^s *s* large (3*D*, 0*d*)	
4 P^s *s* small (4*D*, 0*d*)	petal length 15 to 22.5 mm

In the backcross *s d ʰfranciscana·flavens* × *s d ʰfranciscana* (culture 2288 of 1931) the observed frequencies were:

12 *S* large (8*D*, 4*d*)	petal length 35 to 40 mm
9 *s* large (5*D*, 4*d*)	
8 *s* small (5*D*, 3*d*)	petal length 15 to 22.5 mm

Classification for homozygous *versus* heterozygous P^s was difficult because of the difference in size of the bud cones. Dwarf stature (*d*) segregated independently of flower color and flower size. In the backcross *s d ʰfranciscana·flavens* × *flavens* (culture 2287 of 1931) there were 11 P^s and 10 p, all of which had large flowers.

In this series of crosses, small flower size was confined to classes homozygous for chromosomes 1·2, 3·4 of *ʰfranciscana* (that is, homozygous for *s* carried in 3·4). The backcross to the large flowered *s d ʰfranciscana* showed that small flower size was dominant in plants homozygous for 1·2, 3·4. The gene responsible for small flower size (probably C_o) was independent of chromosomes 1·2, 3·4 since it was associated with them in just 50 percent of the gametes. There must therefore have been a dominant "suppressor" of small flower size carried in 1·4 or 2·3 of *flavens* which prevented

the appearance of small flowers in plants with yellow flowers. Since neither parent had small flowers and since small flower size was dominant when associated with the *h franciscana* chromosomes, it is apparent that the gene for small flowers was introduced into the cross from *flavens*.

The F_1 *sulfurens·flavens* had the configuration 8, 4, 2 (EMERSON 1931b). The ring of 4 involves chromosomes 7·10, 8·9 of *sulfurens* with 7·8, 9·10 of *flavens*, and the pair was either 1·4 or 2·3, if the latter sulfur flowers should segregate independently since *S* is known to be in 2–3 of *flavens* (EMERSON and STURTEVANT 1931). In the F_2 (culture 2282 of 1931) there were 22 plants with large yellow flowers (2 of which had the configuration 8, 2, 2, 2), and there was one plant with large sulfur flowers (also 8, 2, 2, 2). In the backcross *sulfurens·flavens*×*flavens* (culture 2283 of 1931) there were 26 plants with large yellow flowers, 2 of which had the configuration 8, 2, 2, 2 (that is, *sulfurens·flavens*, but homozygous for chromosomes 7·8 and 9·10 of *flavens*). In the backcross *Oe. suaveolens* × *sulfurens·flavens* (culture 2284 of 1931) there were 19 plants (all presumably *albicans·flavens*) with large yellow flowers. In the backcross "*franciscana sulfurea*" × *sulfurens·flavens* (culture 2285 of 1931), 6 plants were of the constitution *s d h franciscana·flavens* with striped buds (one plant had the configuration 4, 4, 2, 2, 2 and one had 4, 2, 2, 2, 2, 2; the extra pairs in the latter must have been 7·10, 8·9), and 6 plants were of the constitution *sulfurens·flavens* with green buds (3 plants had the configuration 8, 4, 2). All plants in this backcross had large yellow flowers.

The backcrosses to "*franciscana sulfurea*" and to *Oe. suaveolens* (*albicans*) were backcrosses for *s* and indicate that the factor pair *S s* was not independent of the ring of 8 in the F_1. The pollen lethal of *sulfurens* and the zygote lethal of *flavens* (in chromosome 5·6) were also in the ring of 8. The pair in *sulfurens·flavens* must have been 1·4 (known to be either 1·4 or 2·3 with *S* in 2·3 of *flavens*). The single sulfur-flowered plant in the F_2 must have arisen from a crossover bringing *s* into *flavens* (see discussion of crossing over for *s* in rings in EMERSON 1931a). Since no small flowered plants appeared in the F_2 or backcrosses, the flower size suppressor of *flavens* must have been in the ring of 8 chromosomes and consequently in chromosome 2·3 of *flavens* (known to be in either 1·4 or 2·3).

An analogous case is perhaps to be found in RENNER's hybrid *flavens·velans*. As in *s d h franciscana·flavens*, the P^s of *velans* is in chromosome 3·4 which together with 1·2 forms a ring of 4 with 1·4, 2·3 of *flavens* (EMERSON and STURTEVANT 1931). Concerning this hybrid RENNER says (1925, pp. 42–43): "Auffällig ist, dass *p-velans* grossere Blüte vererbt als *P-velans*, and entsprechend *P-flavens* kleinere Blüte als *p-flavens*. Mit *P* bezw. *p* sind

also Blütengrössefaktoren gekoppelt, die mit den oben erwähnten selbständig spaltenden (the independently inherited C_o) nichts zu tun haben, und der sehr grossblümige Komplex *velans* kann durch einen Anteil des kleinerblütigen Komplexes *flavens* die Blütengrösse, die er vererbt, noch steigern, und umgekehrt kann *flavens* durch eine Anleihe an *velans* etwas von seiner Blütengrösse einbüssen; durch Austauch der von *P* bezw. *p* nicht abhängig C_o Faktoren wird die Blütengrösse dagegen im umgekehrten Sinn abgeändert." It is almost certain that RENNER'S *p* velans had chromosomes 1·4 and 2·3 from *flavens*, and similarly *P flavens* must have had 1·2, 3·4 from *velans*, so the same "suppressor" of small flower size in chromosome 2·3 of *flavens* may have been responsible for the differences in flower size reported in this cross. One gathers from RENNER'S account that these flower size differences were obtained in out-crosses to *biennis* in which all plants should have had small flowers except those receiving the *flavens* "suppressor."

The same situation arises in the crosses of LANGENDORF, referred to in the preceding section. All backcrosses to plants of large flower size showed segregation for flower size except backcrosses to *flavens* (see summary of LANGENDORF'S data in table 1).

The linkage between flower size and *P* observed by GERHARD (1929) in *acuens* hybrids seems to be somewhat different and may represent the action of a different gene.

INHERITANCE OF *brevistylis*

The inheritance of the *brevistylis* character (b_r) is similar to that of flower size at the C_o locus. DE VRIES (1913) reported that b_r segregated independently of other characters in *Oe. biennis* × *Oe. Lamarckiana brevistylis* F_1 *velutina* (*that is*, ^{b}*albicans*·b_r *velans*). It has since been shown (CLELAND and OEHLKERS 1929) that *albicans*·*velans* had a ring of 14 chromosomes. If the original F_1 of DE VRIES had the same configuration, it appears that b_r was inherited independently of all the chromosomes of the *velans* complex. This appearance is supported by other crosses of DE VRIES: ^{b}*albicans*·b_r *gaudens*, *rigens*·b_r *gaudens*, *rigens*·b_r *velans*, *curtans*·b_r *gaudens*, *curtans*·b_r *velans*, and ^{h}*Hookeri*·b_r *velans*, in all of which b_r segregated independently. Similarly, SHULL (1926, 1928) has found b_r to be independent of all other characters studied by him.

Recent tests of the inheritance of *brevistylis* gave similar results. An F_1 plant from the cross *Oe. R Lamarckiana*×*Oe. Lamarckiana brevistylis* was found to have the configuration 12, 2 (STURTEVANT 1931), the same as in both parents. The gene *R* (red mid-ribs) was known to be in the pairing

chromosome 1·2 (EMERSON and STURTEVANT 1931). The F_2 (culture 14 of 1931) bred true for the characters carried in *velans* and *gaudens* but *R* and b_r segregated (2 *R* B_r, 3 *R* b_r, 2 *r* B_r), indicating that these two genes were independent of the *velans* and *gaudens* complexes (that is, independent of the ring of 12) which is of course in strict agreement with the data of DE VRIES (1900 and later), DAVIS and SHULL for independent segregation of b_r in inbred lines of *Oe.* B_r b_r *Lamarckiana*. The F_1 plant backcrossed to *Lamarckiana brevistylis* gave a progeny of which 88 plants flowered (culture 15 of 1931). All these were typical *Lamarckiana* (that is, *velans*·*gaudens*) but with *R* and b_r segregating independently. The observed frequencies were: 14 *R* B_r, 25 *R* b_r, 19 *r* B_r, and 30 *r* b_r, giving 44 of the parental types and 44 with new combinations. In these crosses b_r was independent of *R* in the pairing chromosome and of all genes in the ring of 12 (for example, P^s *p*, *N n*, the zygotic lethals, etc.).

The hybrid *jugens*·b_r *gaudens* (*Oe. Shulliana* × *Oe.* b_r *Lamarckiana*) was found to have a ring of 14 chromosomes (STURTEVANT 1931) and still b_r was found to segregate independently in F_2 (see discussion of this cross in the following section). It follows that b_r (like c_o) must either lie outside all the fourteen chromosomes or at such a distance from the translocation point in some chromosome as to give 50 percent crossing over with that point.

LINKAGE BETWEEN C_o AND b_r

One of the revolute-leaved mutations of *Oe. pratincola* (BARTLETT 1915) which had relatively small flowers and red mid-ribs (*R*?) was pollinated by *Oe. Lamarckiana brevistylis*, which had large flowers and white mid-ribs. An F_1 plant (*b*$_r$ *velans*) with the configuration 6, 4, 2, 2 gave the following frequencies in F_2 (culture 2314B of 1931):

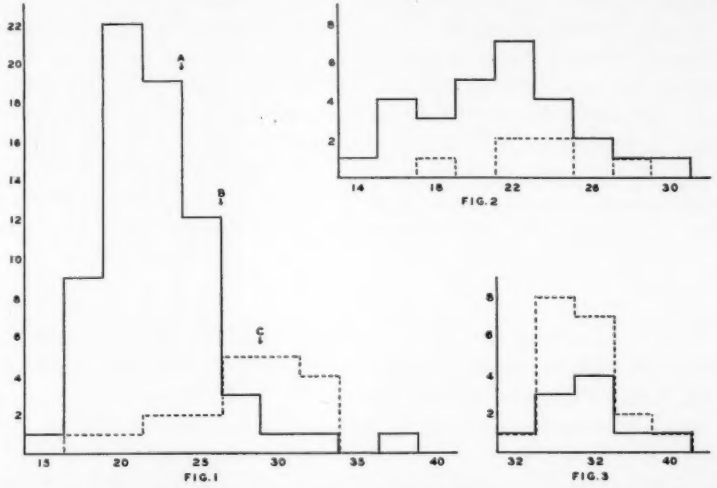
37 flat leaves, red mid-ribs, long styles
13 flat leaves, red mid-ribs, *brevistylis*
25 flat leaves, white mid-ribs, long styled
4 flat leaves, white mid-ribs, *brevistylis*
7 revolute, red mid-ribs, long styles
4 revolute, red mid-ribs, *brevistylis*

The data indicate that revolute leaves (*f*) and red mid-ribs (*R*?) were very closely linked while *brevistylis* (b_r) was completely independent of both.

Petal length varied between 15 and 37.5 mm. The population gave a bimodal curve, indicating a segregation into large and small flowered

types, but there was considerable intergrading between these types. The relation of flower size to *brevistylis* in the F_2 is shown graphically in figure 1.

The mean length of petals for long styled plants was 22.5 mm and for *brevistylis* plants 29.7. The coefficient of correlation between these two characters was 0.51 ± 0.05. The plants were arbitrarily divided into two classes for flower size at the petal lengths indicated in the graph as A, B, and C, and the following linkage values were obtained by use of the tables published by IMMER (1930): at A, 21 percent crossing over; at B 15 percent and at C 17 percent. The closest fit to a 3:1 ratio for flower size is at



FIGURES 1 to 3.—Distribution of flower size in *brevistylis* plants (broken lines) and in normal plants (entire lines) in certain crosses (see references in text). The frequencies (number of plants) are plotted as ordinates, the length of petals in mm as abscissas.

B, indicating that the most probable crossover value was in the neighborhood of 15 percent.

In another cross, however, there was no indication of linkage between flower size and the *brevistylis* character. This was a cross between *Oe. b_r Lamarckiana* and *Oe. Shulliana*. The F_1 (*jugens* · *b_r gaudens*) had a ring of 14 chromosomes (STURTEVANT 1931). In the F_2 (culture 41 of 1931) there were 28 long-styled plants (mean petal length 20.8 mm) and 6 *brevistylis* plants (mean petal length 22.2 mm). The relation of flower size to *brevistylis* in this cross is shown graphically in figure 2. These data, however, are not sufficient to show that no linkage existed in this cross.

The observed linkage with crossing over between flower size (C_o) and *brevistylis* (b_r) eliminates the possibility that these two genes lie outside the chromosomes. It is now almost certain that they are both near the end of one of the chromosomes with about 50 percent crossing over between the translocation point and the nearest gene. SHULL's chromosome II and RENNER's V become identical. Because of the high frequency of crossing over between these genes (C_o and b_r) and the translocation point it is not possible to determine which chromosome these genes are in. It is still possible that they are at the end of one of the established linkage groups.

A test of the direct effect of b_r on flower size was available in the cross between *R Lamarckiana* and b_r *Lamarckiana* reported in the preceding section. The *brevistylis* parent was of the Dutch strain which had slightly larger flowers than the Swedish strain carrying *R*. In the backcross to the larger flowered type (b_r), no difference was found in flower size between long-styled and *brevistylis* plants. Only 10 long-styled plants were measured; these had a mean petal length of 36 mm. Nineteen *brevistylis* plants were measured and found to have a mean petal length of 35.8 mm (figure 3).

SUMMARY

In Oenothera, most genes that were found to be independently inherited in hybrids with pairing chromosomes show nearly complete linkage in hybrids in which the chromosomes are in rings.

Red mid-ribs (*R*) and old-gold flower color (*v*) have been found to be linked and have been demonstrated to be in chromosome 1·2. Similarly, the linked genes *P* (punctate stems, various bud colors), *s* (sulfur flowers) and *n* (*nanella* stature) have been demonstrated to be in chromosome 3·4. In hybrids in which either or both 1·2 and 3·4 appeared as pairing chromosomes, the linkage group *R-v* was found to be inherited independently of the linkage group *P-s-n;* but, in hybrids in which both 1·2 and 3·4 were present in a common ring, these two linkage groups were not independent.

From the association of *d* (dwarf stature) with the ring chromosomes in certain hybrids, and from its independence of the ring chromosomes and of genes in 1·2 and 3·4 in other hybrids, it has been shown that *d* was inherited independently of all chromosomes of *[h]franciscana* except 13·14, but there is no direct evidence placing *d* definitely in that chromosome.

Only two known genes have failed to show the variable linkage relations so universally found in Oenothera hybrids. The gene for small flower size (C_o) and the gene for *brevistylis* (b_r) have been found to be inherited

independently of all other genes, even in hybrids in which all 14 chromosomes were in a single ring. It has been suggested that C_o and b_r lie at such a distance from the translocation points in the chromosomes as to give 50 percent crossing over with these points.

Linkage between C_o and b_r with about 15 percent crossing over has been found, proving that these two genes are in the same chromosome but at a great distance from the translocation point.

The different type of flower size inheritance found in *flavens* hybrids has been accounted for on the basis of a dominant gene in chromosome 2·3 of *flavens* which "suppressed" the normally dominant C_o which was also carried by *flavens*.

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GENES IN MAIZE FOR POLLEN STERILITY

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INTRODUCTION

Studies of a group of genes in maize which have to do with pollen sterility are reported in the present paper. It should be pointed out that the type of sterility reported here is different from the phenomenon of self- or cross-sterility (EAST 1929) which is better characterized by the terms self- or cross-incompatibility (STOUT 1917). In the case of self- or cross-sterility, pollen of a given genetic constitution may or may not be capable of effecting fertilization depending on the genetic constitution of the plant to which it is applied. In other words, the pollen is functionally complete. In the present paper the term sterile is used to designate incompletely developed pollen which is incapable of effecting fertilization under any conditions.

Cases of pollen sterility in maize due to simple genetic factors have been reported. L. A. EYSTER (1921) described a simple recessive which is characterized by abortion of all the pollen. This character was given the name "male sterile" and the genetic symbol m_s. SINGLETON and JONES (1930b) have described a similar character. W. H. EYSTER recently (1931a, b) has reported two additional genes for male sterility. The writer (1930b, 1931, and in press) has studied three genes in maize which result in aberrant chromosome behavior and consequent pollen sterility.

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RHOADES (1931) has studied a type of pollen sterility in maize which is inherited maternally, presumably through the cytoplasm. Pollen sterility is known to result in maize from unfavorable environmental conditions, from chromosome unbalance, from chromosome deficiency and translocations, but these cases are outside the scope of this paper and are therefore only briefly mentioned.

In a number of cases the characters reported here have been studied cytologically. However it should be emphasized that such cytological observations as are mentioned are in no manner complete. It was desired merely to find at what stage of pollen development visible deviations from the normal could be easily detected by the use of simple and rapid methods.

MATERIAL AND METHODS

The pollen sterility characters reported in the present paper are all simple in inheritance, being in most cases differentiated from the normal by single genes. Unless otherwise stated, the characters reported here are simple recessives.

Several recessive pollen sterility characters have been reported in the literature of maize genetics. Since stocks of the male sterile reported by L. A. EYSTER were lost, SINGLETON and JONES (1930) designated the pollen sterility character which they described as male sterile-1 (m_{s1}). W. H. EYSTER (1931a, b) has given two additional male steriles the names male sterile-2 (m_{s2}) and male sterile-3 (m_{s3}). The writer has followed the terminology used by the above mentioned writers for phenotypically similar characters. In the case of pollen sterility characters phenotypically distinguishable from the "male steriles" distinctive names have been used.

The characters discussed in the present paper have been collected from various sources. A list of the various characters considered with the sources from which the writer obtained them is given as follows:

Variable sterile-2 (v_{a2})—Doctor R. A. EMERSON, CORNELL UNIVERSITY, Ithaca, New York. From a yellow dent grown for study of number of kernel rows.

Warty anthers (w_a)—Doctor R. A. EMERSON. From mosaic variegated pericarp strain originally obtained from Czechoslovakia.

Male sterile-1 (m_{s1})—Doctor W. R. SINGLETON and Doctor D. F. JONES, CONNECTICUT AGRICULTURAL EXPERIMENT STATION, New Haven, Connecticut. Reported by SINGLETON and JONES (1930). Male sterile-1 was also found in an inbred strain of white dent obtained from Doctor G. F. SPRAGUE of the UNITED STATES DEPARTMENT OF AGRICULTURE, Washington, District of Columbia.

Male sterile-2 (m_{s2})—Doctor W. H. EYSTER, BUCKNELL UNIVERSITY, Lewisburg, Pennsylvania. Reported by EYSTER (1931a).

Male sterile-3 (m_{s3})—Reported by W. H. EYSTER (1931b). This male sterile has not been grown by the writer.

Male sterile-4 (m_{s4})—Doctor R. G. WIGGANS, CORNELL UNIVERSITY, Ithaca, New York. From an inbred strain of a yellow dent.

Male sterile-5 (m_{s5})—Doctor R. G. WIGGANS. From an inbred strain of the Bloody Butcher variety.

Male sterile-6 (m_{s6})—Doctor R. G. WIGGANS. From an inbred strain of a yellow dent.

Male sterile-7 (m_{s7})—Doctor R. G. WIGGANS. From an inbred strain of a yellow dent.

Male sterile-8 (m_{s8})—Doctor R. A. EMERSON. From a genetic culture. Male sterile-8 was also found in an inbred strain of a yellow dent grown by Doctor R. G. WIGGANS.

Male sterile-9 (m_{s9})—Doctor R. A. EMERSON. Found in the progeny of a cross between Alberta flint and a South American variety. Male sterile-9 was also found in a native yellow dent variety.

Male sterile-10 (m_{s10})—Found in the F_2 generation of a cross between the Cornell-11 variety and a South American variety.

Male sterile-11 (m_{s11})—Found in a yellow dent strain grown by Doctor C. B. HUTCHISON while at CORNELL UNIVERSITY.

Male sterile-12 (m_{s12})—Obtained from Doctor C. R. BURNHAM, National Research Fellow at CORNELL UNIVERSITY. From the F_2 generation of a cross between a brown midrib character and iojap stripe.

Male sterile-13 (m_{s13})—Doctor R. A. EMERSON. From a culture of white rice pop.

Male sterile-14 (m_{s14})—Found in a culture of Argentine flint.

Male sterile-15 (m_{s15})—Doctor M. T. JENKINS of the UNITED STATES DEPARTMENT OF AGRICULTURE and IOWA STATE COLLEGE, Ames, Iowa. From an inbred strain of a yellow dent.

Male sterile-16 (m_{s16})—Doctor M. T. JENKINS. From an inbred strain of a yellow dent.

The writer is grateful to the above mentioned persons who supplied the material for the studies reported here.

Many of the characters reported in the present paper were studied cytologically. These studies were made by means of the aceto-carmine method, a rapid and convenient method for the kind of preliminary survey desired. Material was killed in acetic alcohol (70 percent alcohol and 30 percent acetic acid) and stained with aceto-carmine or a mixture of aceto-

carmine and Ehrlich's haematoxylin as used by COOPER and BRINK (1931). In general, at least 3 plants of any one type were examined. In all cases the several plants of a given character showed essentially the same kind of behavior.

NORMAL POLLEN DEVELOPMENT

In order to describe the deviations from the normal that are found in the various cases of pollen abortion, it is necessary to have in mind the normal sequence of events during the course of pollen development. The meiotic divisions have been described and figured (RANDOLPH 1928, MCCLINTOCK 1929a). As the result of the two meiotic divisions, the microsporocyte is divided into four cells. These cells rapidly increase in size. After several days wall formation begins. Under ordinary growing conditions the microspore nucleus undergoes a vegetative division about a week after meiosis. During this division the chromosomes are large and have prominent constrictions (MCCLINTOCK 1929b). At this time the spore wall is well formed. Approximately a week later and a short time before the pollen is shed, one of the nuclei formed by the division just mentioned divides equationally to give rise to the two sperm nuclei which, at the time the pollen is shed, are crescent shaped. Thus the mature pollen grain contains three nuclei, one larger and more or less spherical and two smaller crescent shaped. Starch formation in the pollen grain takes place during a few days before shedding.

VARIABLE STERILE-2

Description

After the character variable sterile (v_a) was described (BEADLE in press) another character was found which is somewhat similar. It has been given the name variable sterile-2 and the symbol (v_{a2}).

Variable sterile-2 plants can be distinguished in the field by the fact that they shed but little pollen. Usually anthers are exserted but not systematically as they are in normal plants. The exserted anthers are usually rather sparsely scattered through the inflorescence. The anthers contain a variable amount of apparently good pollen. Anthers containing a high percentage of good pollen may dehisce and shed. However, most of the anthers fail to shed their contents presumably because there is insufficient pressure within the anther to cause the normal splitting of the suture at the tip of the anther. Variable sterile-2 plants are apparently completely female fertile.

Inheritance

Variable sterile-2 is a simple recessive character. As originally found, it appeared in about 25 percent of the plants. A v_{a2} plant crossed with homo-

zygous normal gave 12 normal plants. These backcrossed to v_{a2} plants gave a ratio of approximately 1 V_{a_2} to 1 v_{a_2}. A few progeny were grown from selfed seed of heterozygous variable sterile-2 plants. These were classified as 45 V_{a_2} and 14 v_{a_2}. Though the numbers are small, this is a close approximation to the expected 3:1 ratio. From crosses of v_{a2} by V_{a2} v_{a2} plants, 135 plants were grown. Among these, 72 were normal and 63 v_{a2}. This is in fairly good agreement with expectation. In all, the v_{a2} character has been under observation through 5 generations, and, although the num-

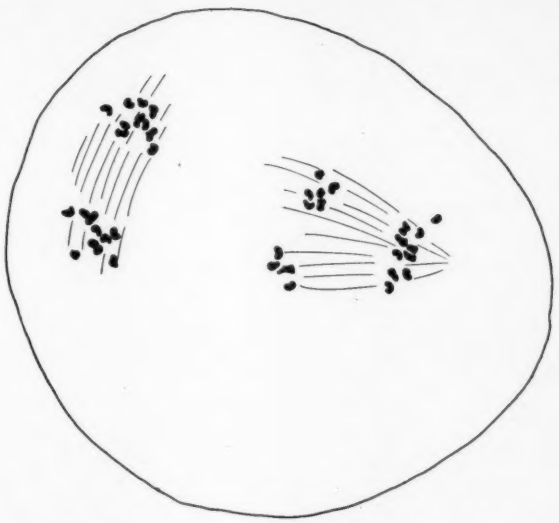

FIGURE 1.—Anaphase of the second meiotic division in v_{a2} plant in a cell in which cytokinesis failed to take place during the first division. There are two spindles and the distribution of chromosomes is apparently normal. (×775)

bers grown have been small, no significant departures from the expected behavior have been observed.

Cytological observations

Meiotic chromosome behavior was studied in 4 variable sterile-2 plants and was found to be somewhat similar to that of variable sterile-1 (BEADLE in press). Chromosome behavior seems to be quite normal during the course of the first division. Diakinesis, metaphase, anaphase and telophase stages have been observed. As in v_{a1} plants a variable percentage of the

microsporocytes fail to undergo cytokinesis during division I. Five to ten percent might be considered a fair estimate of the percentage of micro-

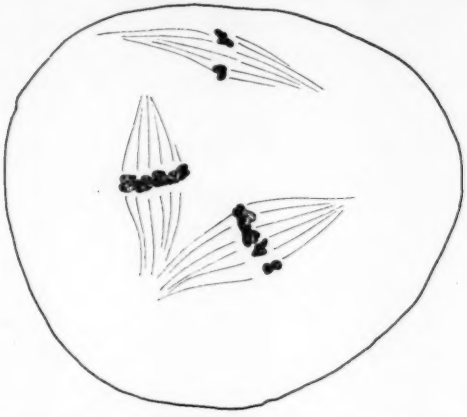

FIGURE 2.—Metaphase II in a v_{a2} plant in which there was no cytokinesis during division I. Three spindles are evident. (×775)

sporocytes which show such behavior. In the cells which divide normally during the first division, the second division is usually normal and pro-

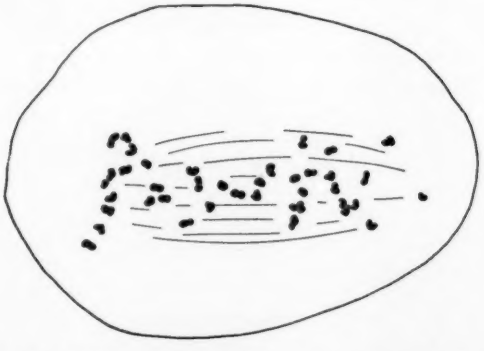

FIGURE 3.—Anaphase II in a v_{a2} plant in which there was no cytokinesis during division I. There is much lagging of chromosomes. (×775)

duces a normal appearing group of four spore cells. In the cells in which cytoplasmic division fails to take place during division I, the second divi-

sion is often aberrant. Two spindles each containing ten chromosomes can be observed in some cells (figure 1). In other cells more than two spindles can be seen (figure 2). In still other cells only one spindle may be formed. In such cases the chromosome distribution may be fairly regular, twenty chromosomes going to each pole, or there may be a great deal of lagging which results in an unequal distribution (figure 3). In variable sterile-1 plants it was found that cytokinesis usually fails to take place during the second division in those cells in which it fails during the first division. In contrast to this behavior, cytokinesis often takes place in the second division in variable sterile-2 plants in cells in which it failed during the first division. Groups of variable numbers of unequal-sized spore cells may result from aberrant second divisions (figure 4). In such cells as are illustrated in figure 3 in which there is a great deal of lagging of chromosomes, the division is usually not completed. Often no metabolic nuclei are formed, the cells degenerating with the chromosomes in the condensed

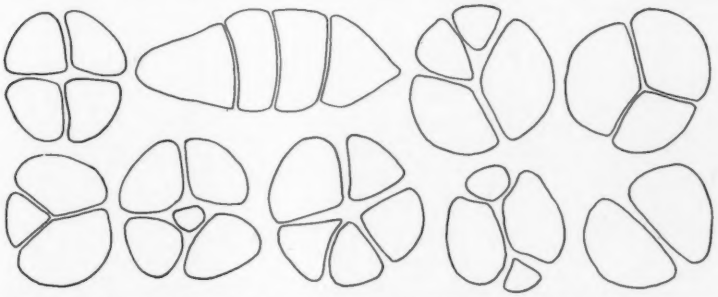

FIGURE 4.—Groups of spore cells from a v_{a2} plant showing variations in number, arrangement and size.

form. During the degeneration, the individual chromosomes form spherical vesicles of weakly staining chromatin.

In many of the spore cells in variable sterile-2 plants there is an apparent tendency toward a precocious division. Very soon after the second meiotic division is completed the chromatin begins to change from the typical metabolic form and assume the form of visibly identifiable chromosomes. This prophase-like condition is very similar to the prophases observed in polymitotic maize plants during the first supernumerary division of the spore cells (BEADLE 1931). Apparently the spore cells in v_{a2} plants do not go beyond the prophase stage of a division. They usually degenerate in a rather early prophase-like condition. In a few cells the chromosomes condense to a stage such as is illustrated in figures 5 and 6,

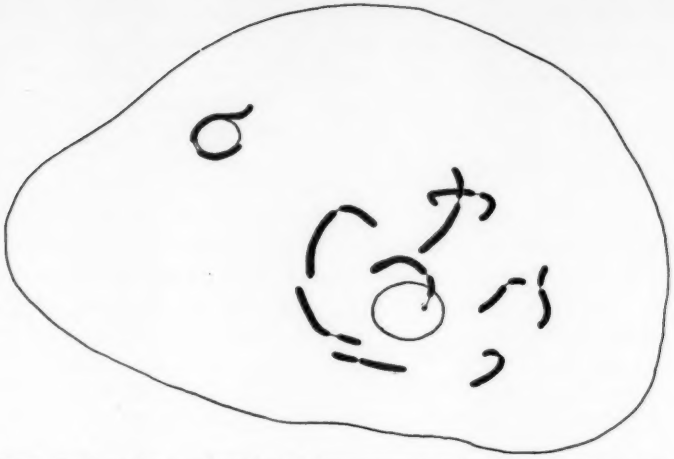

FIGURE 5.—Precocious condensation of chromosomes in a haploid spore cell of a v_{a1} plant. (×1560)

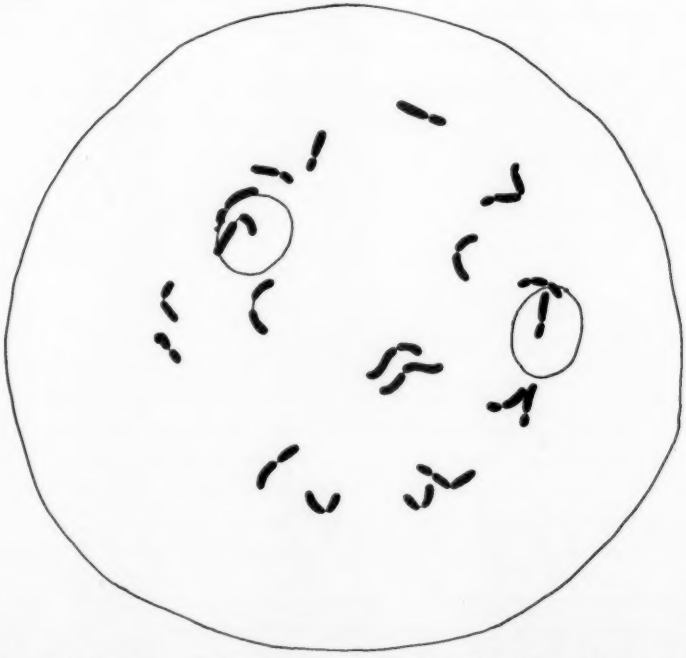

FIGURE 6.—Precocious condensation of chromosomes in a diploid spore cell of a v_{a1} plant. (×1560)

both diploid and haploid cells showing this tendency (figures 5 and 6). The constrictions are very prominent in these prophase-like stages. The relative lengths of the chromosomes and the position of the spindle attachment constrictions is clearly evident in such cells (figure 5). In general these stages are similar to the prophase stages of the first vegetative division of the microspore nucleus (McCLINTOCK 1929b).

The diploid microspore cells which show a tendency to undergo a precocious division are interesting in that the chromosomes show no visible split and do not pair, as might be expected on the basis of DARLINGTON'S (1931) theory of synapsis. However, the chromosomes might well be

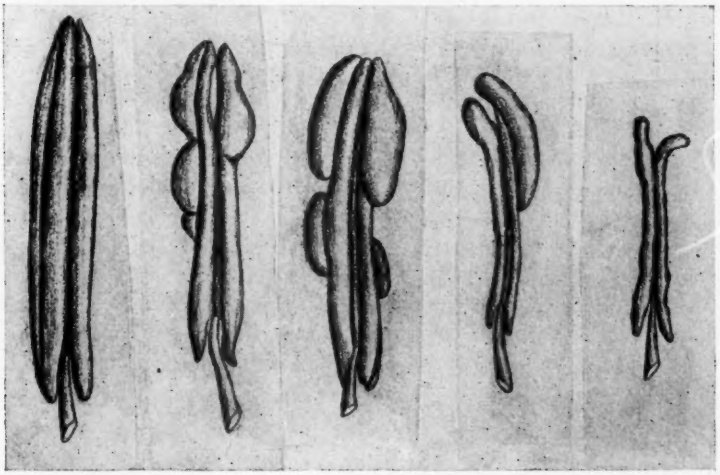

FIGURE 7.—Anthers of a normal (to the left) and a warty anther plant (4 anthers to the right) showing the characteristic shape and the variability of anthers from w_a plants.

actually but not visibly split which would prevent pairing on DARLINGTON'S theory. Furthermore these cells must be abnormal since they very soon degenerate. The case is therefore not a critical test of DARLINGTON'S theory.

Variable sterile-2 plants resemble variable sterile-1 plants in their meiotic chromosome behavior. The apparent tendency of the spore cells to undergo a precocious division is suggestive of the condition observed in polymitotic maize plants.

WARTY ANTHERS

Warty anther plants usually exsert very few anthers and shed little or no pollen. The anthers are very much shriveled except in certain regions which develop normally. The anthers have a very characteristic appearance (figure 7). Very few of the anthers dehisce. However if the tip of the anther is well developed dehiscence may take place and pollen be shed. Tests have shown that at least some of this pollen is functional. Warty anther plants are completely female fertile.

The warty anther (w_a) character is inherited as a simple recessive.

An examination of the young anthers of w_a plants shows that in the shriveled parts of the anther the sporogenous cells degenerate very early. The exact time of degeneration with reference to meiotic stages was not established. It is possible to say, however, that this degeneration begins somewhere near the time of synapsis. In maize synapsis takes place a relatively long time before metaphase of the first division. At this stage the anthers are usually less than one mm long. In the very young anthers of w_a plants the swellings which mark the regions in which the sporogenous tissue does not degenerate are evident. By the time the chromatin threads begin to loosen up after synizesis the cells are in an advanced stage of disintegration. In some anthers all of the sporogenous tissue may degenerate. In others only the tissue in certain sections of the anther degenerates while that in other sections develops normally and gives rise to normal pollen grains. It is difficult to understand why the tissue in certain parts of an anther should so completely break down while adjacent tissue develops quite normally.

MALE STERILES-1 TO -16

Male steriles are of relatively frequent occurrence in maize. The writer has collected such characters from over thirty sources. Doctor M. T. JENKINS (unpublished) has collected male steriles from eighteen sources. Only a representative group of the available male steriles is considered in the present paper.

All of the characters to which the name male sterile has been given are similar in that they are partially or completely pollen sterile and completely egg fertile.

Male sterile-1 (m_{s1})

Male sterile-1 was reported by SINGLETON and JONES (1930). It is a simple recessive character. The gene concerned is located in the V-P_l chromosome within a few crossover units of the V (yellow endosperm) gene. It is not known whether m_{s1} lies in the direction of the P_l (purple

plant color) gene, or in the opposite direction. Male sterile-1 plants may exsert a few scattered anthers but no pollen is shed.

Cytological examinations of m_{s1} plants by the writer show that the two meiotic divisions are completed in a normal manner. The spore cells grow to almost normal size, develop a wall but degenerate before the nucleus goes through the first division and before starch formation begins.

Male sterile-2 (m_{s2})

Male sterile-2 was reported by Doctor W. H. EYSTER (1931a). EYSTER showed that the m_{s2} gene is located in the chromosome which contains, among others, the well known genes *C* (aleurone color), s_h (shrunken endosperm) and w_x (waxy endosperm). The m_{s2} gene gives about 23 percent of crossing over with s_h but it is not known on which side of s_h it lies.

No cytological examinations of m_{s2} plants have been made by the writer.

Male sterile-3 (m_{s3})

Male sterile-3 was reported by W. H. EYSTER (1931b). It was found to be linked with the c_r (crinkly leaves) gene with about 30 percent of crossing over. Its position is not known with respect to other genes in this chromosome which contains a_1 (aleurone and plant color), t_{s4} (tassel seed-4), d_1 (dwarf-1) and others (BRINK and SENN 1931).

EYSTER reports that the anthers of m_{s3} plants contain many walled starchless microspores which have a single nucleus. In a few cases two nuclei were found. It seems, therefore, that degeneration takes place in m_{s3} plants, for the most part, before the microspore nucleus goes through the first division.

Male sterile-4 (m_{s4})

Male sterile-4 is a completely pollen sterile type which does not exsert anthers.

Examination of the anthers of m_{s4} plants established that the two meiotic divisions are normal. Pachytene, diakinesis, metaphase I, telophase I, prophase II, metaphase II and anaphase II stages were observed. A few of the spore cells show the beginnings of wall formation but most of them degenerate before any wall formation is detected.

Male sterile-5 (m_{s5})

Male sterile-5 plants are completely pollen sterile and do not exsert anthers.

Cytological studies show that the meiotic divisions are normal. Cells in pachytene, diplotene, diakinesis, telophase I, prophase II, metaphase II and anaphase II stages were observed. The microspore cells reach the

stage of wall formation, but the nucleus probably does not undergo the first vegetative division. This latter point was not established for certain.

Male sterile-6 (m_{s6})

Male sterile-6 appears to be a simple recessive though because of the variability of the character this has not been established for certain. It is a partially sterile type. Usually the exserted anthers are sparsely distributed through the inflorescence though occasionally anther exsertion is practically normal. The tips of the anthers tend to be empty and therefore have a pinched appearance (figure 8). Such anthers do not dehisce, presumably because of insufficient pressure at the tip where the split in the suture normally occurs. Many anthers contain abundant pollen and in certain plants a small amount of it may be shed.

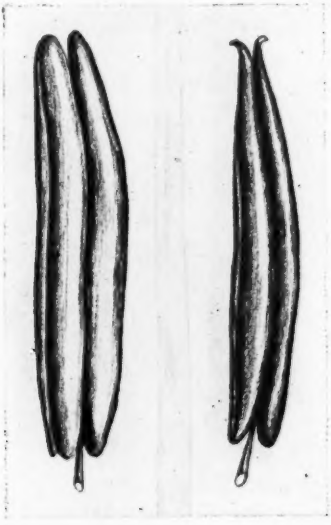

FIGURE 8.—Anther from a normal (left) and from an m_{s6} plant (right) showing the pinched tip characteristic of the anthers of m_{s6} plants.

Cytological examinations of m_{s6} plants show considerable degeneration of microsporocytes before, during and after metaphase of the first meiotic division. The two meiotic divisions are completed in a normal manner in many cells. Apparently a variable percentage of cells degenerate in m_{s6} plants in any one of several stages ranging from before metaphase I to pollen maturity.

Male sterile-7 (m_{s7})

Male sterile-7 is completely pollen sterile and no anthers are exserted. The character is inherited as a simple recessive.

A study of microsporogenesis in m_{s7} plants reveals that the two meiotic divisions are normal. Pachytene, early and late diakinesis, metaphase I, anaphase I, metaphase II, and anaphase II stages were examined. Degeneration of the spore cells takes place after wall formation but probably before the first division of the microspore nucleus. The contents of the anthers are almost completely disintegrated by the time pollen is shed by normal sib plants.

Male sterile-8 (m_{s8})

Male sterile-8 plants are completely pollen sterile and exsert no anthers. The anthers are very much shriveled and their contents have completely degenerated by the time pollen is shed by normal sibs.

Meiosis is rarely completed in m_{s8} plants. Degeneration of the microsporocytes takes place during pachytene or later stages of meiosis. The cytoplasm disintegrates before the chromatin. A few microsporocytes complete meiosis but the resulting spore cells degenerate very soon thereafter. In many of the cells which complete meiosis there is a failure of cytokinesis during the first division and large cells with 40 chromosomes similar to those found in v_{o1} and v_{o2} plants are found. However this behavior in m_{s8} plants differs from that in variable sterile plants in that the cells concerned in m_{s8} plants show distinct signs of degeneration.

Male sterile-8 has been tested with a considerable number of characters in known chromosomes but no good indication of linkage was found. Most of the data were from F_2 cultures. The characters with which m_{s8} was tested and the number of individuals classified are as follows:

s_h	110	f_1	534	P	209	a_1	855
w_x	110	B	1129	b_r	357	t_{s4}	550
R	304	l_g	857	f	273	d_1	380
g_1	1178	Y	822	r_a	408	c_r	427
s_u	348	P_1	1022			P_r	575
T_u	144						

The m_{s8} gene probably lies at the untested end of the r_a or of the P_r chromosome or in the linkage group which probably contains the C_h (chocolate pericarp) gene (ANDERSON and EMERSON 1931).

Male sterile-9 (m_{s9})

Male sterile-9 plants are completely pollen sterile and exsert no anthers.

Cytologically m_{s9} was found to be very similar to m_{s8}. Degeneration takes place during the same stages and apparently in much the same way.

Male sterile-10 (m_{s10})

Male sterile-10 plants are completely pollen sterile and exsert no anthers.

Meiosis is normal in m_{s10} plants. Pachytene, diplotene, metaphase I, anaphase I, metaphase II, and anaphase II stages have been examined. Visible signs of degeneration of the microspore cells make their appearance at about the time of wall formation.

Male sterile-11 (m_{s11})

Male sterile-11 is a completely pollen sterile type that does not exsert anthers. It has not been examined cytologically.

Male sterile-12 (m_{s12})

Male sterile-12 is a simple recessive character that is not completely pollen sterile. Some plants exsert a few anthers some of which dehisce and shed pollen. It has not been examined cytologically. Since m_{s12} is partially sterile it may not be of the same type as the other characters described under the name "male sterile."

Male sterile-13 (m_{s13})

Male sterile-13 plants are completely pollen sterile and exsert no anthers.

Male sterile-13 plants show normal chromosome behavior at meiosis. Very soon after meiosis the spore cells show signs of degeneration. A few cells may reach the stage of wall formation.

Male sterile-14 (m_{s14})

Male sterile-14 is completely pollen sterile and exserts no anthers.

Meiosis is normal in m_{s14} plants. The microspore cells degenerate after wall formation and probably before the first division of the microspore nucleus.

The ms_{14} gene was found to be linked with the s_b (slit leaf blade) gene (BEADLE 1930a). Evidence was presented showing that these two genes are located in the Y-P_l linkage group. Since the above cited paper was written some additional data involving the s_b, Y and P_l genes have been obtained. These data are not sufficient to be conclusive but do not substantiate the earlier data in indicating the s_b is linked with Y and P_l. The conclusion that m_{s14} is located in the Y-P_l chromosome is therefore somewhat doubtful.

Male sterile-15 (m_{s15})

Male sterile-15 is a recessive character obtained from Doctor M. T. JENKINS. Most of the material involving this character grown by the writer has been heterozygous and it is therefore not possible to describe it in any detail. It has not been examined cytologically.

Male sterile-16 (m_{s16})

This character, a simple recessive, was also obtained from Doctor M. T. JENKINS. It is a completely pollen sterile type that does not exsert anthers. It has not been examined cytologically.

OTHER MALE STERILES

A number of male steriles other than those considered above have been studied. Some of them are simple recessive characters but are not considered for the reason that intercrosses have not been completed. Others gave indications of a more complex type of inheritance. In two cases ratios suggested that duplicate recessive genes were concerned. However, these cases were not satisfactorily analyzed as it was decided to study the simpler cases first.

Cytological studies of a simple recessive male sterile obtained from one of Professor R. A. EMERSON'S linkage testers showed that the microsporocytes degenerate during synizesis or pachytene stages. This behavior differs from that of any of the other male steriles which have been studied cytologically and it is probable therefore that the gene concerned is different. However, this male sterile may be the same as some of those considered above which were not examined cytologically. It has not been intercrossed with other male steriles.

INTERCROSSES OF POLLEN STERILE CHARACTERS

Since most of the characters under consideration are completely pollen sterile, it is necessary in intercrosses to use as pollen parents plants heterozygous for the desired genes. In many cases heterozygous plants were also used as female parents. In all cases the female parent of heterozygous plants used in intercrosses was homozygous for the m_s gene under study.

The data from the intercrosses which have been made are presented in table 1. With the exception of m_{s3}, which the writer did not have, the intercrosses of the male steriles are practically complete. The strain of m_{s13} used was found to be cross sterile as female parent with certain unrelated strains. This behavior is probably the same as that reported by DEMEREC (1929) and explains the absence of intercrosses of m_{s13} with m_{s12}, m_{s15} and

m_{s16}. Variable sterile-1 may not be different from m_{s12} or m_{s15}. With the above mentioned exceptions the data show that the m_s genes concerned are non-allelomorphic.

TABLE 1

Intercrosses of v_{a_1}, v_{a_2}, w_a and male steriles–1 to –16. B indicates normal progeny from a cross between plants homozygous and heterozygous for m_s genes ($m_{sx}\ m_{sx} \times M_{sy}\ m_{sy}$). F indicates normal progeny from a cross between plants heterozygous for m_s genes ($M_{sx}\ m_{sx} \times M_{sy}\ m_{sy}$). C indicates phenotypic of cytological difference. L indicates that genes are located in different chromosomes.

	ms_{16}	ms_{15}	ms_{14}	ms_{13}	ms_{12}	ms_{11}	ms_{10}	ms_9	ms_8	ms_7	ms_6	ms_5	ms_4	ms_3	ms_2	ms_1	wa	va_2
va_1		C	C	C		C	86F	257F	95B	106F	89F	60B	77F	CL	CL	57B	40F	53F 54F
va_2	11B	21B	70F 31B	37F	37B	39B	56F 41B	139F 144B	174F 40B	88F	90F	99F	84F	C	38B	58B	56F	
wa	38B	*36F	104F	30F	36F	40B	100F	242F	101B	53B	53F	83B	67B	C	55F	52B		
ms_1	36B	36B	62B	39B	40B	68B	56B	202B	40F 136B	55B	50B	56B	56B	L	84B			
ms_2	34B	20B	38F 45B	46F	30B	38F	26F 31B	43F	67F 72B	70B	75F	69B	57B	L				
ms_3											C							
ms_4	20B	8B	90F	33B	39B	39B	114F	173F	137F 14B	88F	94F	85F						
ms_5	9B	24B	78B	42B	49B	45B	78B	143F 120B	141F 40B	94F	47F							
ms_6	21B	30B	101F	42F	38F	38F	116F	391F	187F	46F								
ms_7	29B	22B	56B	44B	20B	45B	96F	197B	172F 42B									
ms_8	35F 41B	44F 20B	40F 79B	29F	30F 30B	39B	40F 83B	70F 45B										
ms_9	51B	37B	316F	43B	51B	45B	214F											
ms_{10}	40B	17B	94F	37F	41F	36B												
ms_{11}	43F	38F	33F	34F	39F													
ms_{12}	22B	20B	39F															
ms_{13}			49F															
ms_{14}	12B	13B																
ms_{15}	24F																	

*One m_s plant observed in this cross. Possibly the result of an accidental self pollination of the female parent.

DISCUSSION

As has already been stated, no attempt has been made in the present paper to describe in detail the disintegration of the sporocytes or microspore cells in the various sterile types studied. Such a study would of course require the use of more refined methods than any that were used in the

course of the present study. In general the cytoplasm showed changes before any deviation from the normal was detected in the chromatin. The nature of the changes in the cytoplasm was not studied in detail but in advanced stages of degeneration it is completely broken down. In the sporocytes it tends to break away from the main body of the cell. In the walled microspores it breaks down completely, the amount of stainable cytoplasm being gradually reduced. The chromatin tends to accumulate in spherical droplets which appear to have a watery consistency during the late stages of the process of disintegration.

In most of the characters studied all the microsporocytes or microspore cells show the same changes at about the same time. Exceptions to this were observed in warty anther plants where the degeneration is confined to certain regions of the anthers and in male sterile-6 plants where the degeneration seems to take place at any stage over a comparatively wide range.

The question of what changes occur in the tapetal cells which are generally assumed to have a nutritive function is important. Some observations were made on these cells but the methods were unsuited to such a study and the observations themselves so limited in extent that it is not considered worth while to discuss them in more detail than to say that in many of the characters tapetal tissue was seen to be disintegrating at the time at which the anther contents were undergoing degeneration.

It seems reasonable to assume that degeneration takes place in the cells under consideration in male sterile plants because of some sort of nutritive disturbance.

Judging from the number of genes which affect them the processes of microsporogenesis and pollen development must depend on easily disturbed physiological conditions. The case is perhaps comparable to chlorophyll development which is likewise known to be frequently disturbed by genetic changes.

Genetic pollen abortion appears to be of much more common occurrence than megaspore or egg abortion, no cases of the latter unaccompanied by the former having to the writer's knowledge been reported. (In the character, silkless, reported by JONES [1925] and ANDERSON [1929], RANDOLPH [unpublished] has shown that the entire pistil aborts early in development. Anthers develop in the pistillate inflorescence of silkless plants. The character is comparable to the tassel seeds [EMERSON 1920] in which the stamens abort early in development and permit the development of pistils in the inflorescence that would normally be staminate.) The female sporocytes and spore cells probably have an advantage of position, as they are

imbedded in a rather large mass of sporophytic tissue during the entire course of their development.

SUMMARY

A study of fifteen genes for pollen sterility characters is reported. In all of these characters the development of megaspores and female gametophytes is normal.

In the character variable sterile-2 (v_{a2}) as in variable sterile-1 (previously reported), cytokinesis often fails to be completed during meiosis. The young spore cells in v_{a2} plants apparently have a tendency to go through precocious post meiotic division. The chromatin condenses as prophase-like chromosomes which show no signs of splitting. The cells degenerate without showing further progress of division.

A gene to which the name "warty anther" and the symbol w_a has been given is reported. In w_a plants the very young microsporocytes degenerate in certain regions of the anther but develop normally in other regions.

Thirteen additional genes for male sterility are reported. These have been given the names male sterile-4 to -16 and the symbols m_{s4} to m_{s16}. Intercrosses show that with one possible exception the genes concerned in the production of these male sterile characters are non-allelomorphic.

Cytologically the male steriles are characterized by degeneration of the microsporocytes or of the microspore cells. The time of degeneration may be the same or different in different male steriles and ranges from the synizetic stage of meiosis almost to pollen maturity.

In no case are the linkage relations known for the male steriles reported for the first time in this paper.

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GENETIC AND CYTOLOGICAL INVESTIGATIONS ON *ASPARAGUS OFFICINALIS* L.[1]

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INTRODUCTION

The purpose of this investigation was to study variation and inheritance in *Asparagus officinalis* with especial emphasis on sex from an experimental and cytological standpoint. For the most part former scientific investigations on this species have had to do with some phase of its commercial production, such as culture, disease, quantity of production, etc. SHOJI and NAKAMURA in 1928 described their observations, mostly histological, on the degeneration of the flower parts of one sex in the flowers of the opposite sex. A somewhat similar paper by LEVITSKY had already (1925) dealt with the phenomenon of abortion in the flower parts.

ROBBINS and JONES (1925) described several secondary sex characters in this species. The characters described were of a quantitative nature. NORTON (1913) found that rust resistance was usually dominant to susceptibility to rust in asparagus. BERNATSKY (1914) and CORRENS (1928) have described several types of sexual dimorphisms in *A. officinalis*.

[1] Contribution from The Miller School of Biology and The Blandy Experimental Farm, UNIVERSITY OF VIRGINIA.

MATERIAL

The material used in this investigation consisted of plants of varieties Mary Washington, Bonvallet's Giant, Palmetto, Argenteuil, Barr's Mammoth, Giant Emperor, and Conover's Colossal of *Asparagus officinalis*. These plants were raised from seed or from one or two year old crowns secured from commercial seed houses. Most of the seed and crowns came from WILLIAMS SEED COMPANY of Norfolk, Virginia, or from VAUGHAN'S SEED STORE. All observations mentioned in this paper were made upon the variety Mary Washington unless otherwise noted.

HISTORY OF *ASPARAGUS OFFICINALIS*

The native home of *A. officinalis* is in the eastern part of Europe, especially in the vicinity of the Caucasus Mountains and in Siberia, according to STURTEVANT (1890). In the earlier literature CATO and other Romans, as well as Greeks, spoke of asparagus both as a medicinal and as a food plant. From all reports it seems certain that asparagus has been used and cultivated as a food plant for at least two thousand years. Today, *A. officinalis* is found growing wild in various parts of Europe. In such a state it is especially abundant in different sections of Russia.

Approximately one hundred species of asparagus are reported from the old world (ENGLER and PRANTL 1888). Of these *A. officinalis* is the most important economically and the only one cultivated for food. Several other species are grown for ornamental purposes.

According to JONES and ROBBINS (1928) the majority of our present varieties of edible asparagus have been developed within the last five or six decades. Purple Dutch—developed in the eighteenth century—gave rise to the Argenteuil varieties. The Argenteuil varieties originated at the town of that name in France in 1860–1862.

It seems certain that either or both of the above mentioned varieties were introduced into America by early colonists, and that other varieties now grown commercially have had their origin from them. Varieties Conover's Colossal, Palmetto, and Columbian Mammoth White were introduced between 1882 and 1893. Few distinguishing characters are found to separate the several dozen so-called varieties. NORTON (1913), ILOTT (1901), and RIDGEWAY (1909) all point out the decided lack of differential characters between the varieties of asparagus. The Washington varieties are the most recently developed sorts. They have originated from the work of J. B. NORTON. The most generally popular of these varieties is the Mary Washington because of its resistance to rust and because of its more generally uniform size and color.

A. officinalis is a species comparatively free from mutations. The literature on its history does not indicate that the edible asparagus of several thousands of years ago differed very materially from the type that we have today. The variations that are observable in asparagus are probably more of a quantitative than of a qualitative nature. Rust resistance seems to be one of the most clear cut and striking of these differentiations.

SUITABILITY FOR GENETIC AND CYTOLOGICAL STUDY

From the breeding standpoint asparagus is rather unsatisfactory for genetic studies. In the first place the slight amount of varietal or intervarietal variation in *A. officinalis* furnishes few character differences for inheritance studies. Further, between seed harvest and the time of flowering of plants of the next generation there is an interval of two and sometimes three years, in this section of the country. This is a serious disadvantage; and, finally, the few species of asparagus so far found adaptable to natural conditions in this country are, here, a deterrent to genetic studies on the genus as a whole.

As material for a study of the problems of sex this species is more promising. It is dioecious. Hence the proportion of one sex to the other, the constancy of each sex, the general sex behavior, especially in its relations to various environments, as well as the cytological phenomena, involve many interesting and important questions.

This species, because of its relative freedom from mutation, its comparative ease of cultivation and propagation, its long life tenure, its type of fruiting structures, and their indeterminate development habit, should make excellent material for a study of the effect of X-rays and radium on mutation.

Asparagus also lends itself satisfactorily to cytological studies. There is relatively a large amount of chromatin material present, and the chromosomes are relatively few in number. These facts make it possible to observe more or less readily the chromosomes at all stages.

FIELD INVESTIGATIONS

On sexual dimorphisms

Dimorphisms between plants of different sexes of the same species, while much rarer in the plant than in the animal kingdom, have been described from most of the dioecious plants that have been the subject of any intensive sex study. The literature dealing with such characters has been adequately reviewed by ROBBINS and JONES (1925), CORRENS (1928), and YAMPOLSKY (1930), and will not be discussed here.

Casual observations on *A. officinalis* in the field do not reveal any striking vegetative dimorphisms between the two sexes. Although careful studies have revealed several important quantitative differences between staminate and pistillate individuals of the species, the sex of a certain plant cannot be absolutely determined except by examination of its flowers.

BERNATSKY (1914) reported that in *A. officinalis* the staminate plants usually have seven cladophylls per cluster while the pistillate plants have only three to five cladophylls in a cluster. He observed further that the staminate plants have thicker ramifications than the pistillate plants. CORRENS (1928) took careful data on these characters and obtained results which "contradict completely the data of BERNATSKY'S; the experimental males had a mean of fewer cladophylls per cluster, and these were on the average somewhat longer."

ROBBINS and JONES (1925) observed that the staminate plants have a tendency to express their sex earlier in life than the pistillate plants. This is substantiated by the works of CORRENS (1928) and of SHOJI and NAKAMURA (1928), and also by my own observations.

Other quantitative secondary sexual characters reported by ROBBINS and JONES are: (1) The first flower bearing shoots of pistillate plants are higher than the first flower bearing shoots of staminate plants; (2) during the first several seasons of growth the staminate plants yield more stalks per crown than the pistillate ones; and (3) staminate plants outyield pistillate individuals through the first cutting season.

NORTON (1913) noted that the perianth lobes are longer in staminate flowers than in pistillate ones. I have found that the perianth of staminate flowers varies from 5 mm to 7 mm in different plants, while in pistillate flowers the perianth is from 3 mm to 4 mm in length.

Among my plants a noticeable difference has been observed between the buds of the different sexes. Buds which eventually develop into pistillate flowers are pyramidal or wedge shaped, and are more or less pointed at the tip. On the other hand, buds which later develop into staminate flowers are blunt at the tip end, and are more cylindrical in shape. I noticed also that when plants are in full bloom the opposite sexed plants are different in the color cast caused by the flowers. At a casual glance the pistillate plants appear lighter green than is usual for them, due to the whiteness of their flowers. In contrast staminate plants in full bloom have a slight yellow cast due to the large amount of pollen present.

Hybridization

The most important hybridization work on asparagus was that of

NORTON (1913) who collected many strains of *A. officinalis* at Concord, Massachusetts and selected plants for breeding in an attempt to produce a rust resistant type. He mentions the strains that he used but in only a few cases are the results of his experiments given. Attempts to cross the ordinary edible strains of asparagus and *A. officinalis pseudoscaber* failed to give hybrid plants. *A. davuricus*, a related form from China, when pollinated with *A. officinalis* pollen gave seedlings that showed hybrid characters. OLIVER (NORTON 1913) tried to cross *A. virgatus* with *A. officinalis* but without success.

In the present investigation six of the most distinct and most popular varieties of *A. officinalis*, that is, Mary Washington, Bonvallet's Giant, Argenteuil, Giant Emperor, Barr's Mammoth, and Palmetto, have been the subject of hybridization experiments.

Eight varietal crosses, two of them reciprocal, have been made. In these cases a number of seeds or good "seed-sets" have been secured from crosses between at least three different pairs of plants. The crosses are:

Mary Washington ♀ × Palmetto ♂
Mary Washington ♀ × Bonvallet's Giant ♂
Mary Washington ♀ × Barr's Mammoth ♂
Mary Washington ♀ × Argenteuil ♂
Mary Washington ♀ × Giant Emperor ♂
Palmetto ♀ × Mary Washington ♂
Palmetto ♀ × Barr's Mammoth ♂
Giant Emperor ♀ × Mary Washington ♂
Giant Emperor ♀ × Bonvallet's Giant ♂
Giant Emperor ♀ × Palmetto ♂

A number of other intervarietal crosses were made which apparently resulted in seed sets, but which were insufficient in number to assure the validity of the cross. None of the varieties worked with have given other than positive indication of their ability to cross with the other varieties used. The results indicate that with the six varieties used intervarietal crosses are almost, if not quite, as easy to obtain as crosses between plants of the same variety.

Sex ratios

The ratios of staminate and pistillate individuals have been reported in a number of species of dioecious plants. HEYER (1884) (from YAMPOLSKY 1919) found a ratio of 100 staminate to 114.93 pistillate in 40,000 hemp plants, and 106 staminate to 100 pistillate in 21,000 *Mercurialis annua* plants. FISCH (1887) (from YAMPOLSKY 1919) found a ratio of 100 stam-

inate to 154.23 pistillate individuals in 66,327 hemp plants. STRASBURGER (1900) found a ratio of 100 staminate to 128.16 pistillate in 10,662 plants of *Melandrium album*. MCPHEE (1925) found a ratio of 100 staminate to 121 pistillate in 1959 hemp plants.

In asparagus ROBBINS and JONES (1925) found an almost exact 1:1 ratio of staminate and pistillate individuals in 1600 plants. MALHOTRA (1930) observed that in 34,040 Mary Washington plants 28.97 percent were staminate, 60.19 percent were pistillate, while 11 percent were non-blooming; in 7,629 Palmetto asparagus plants 33.1 percent were staminate, 58.5 percent were pistillate and 8.4 percent were non-blooming.

MALHOTRA was able to increase the percentage of pistillate plants by aging pollen before it was used in pollinations. Contrarily he increased the weight of pollen used by spraying it with volatile oil from other pollen and thus increased the percentage of staminate plants produced.

During the summers of 1929 and 1930 the writer observed 2520 asparagus plants for sex expression. In 1929 a close check was kept upon the plants that bloomed until June 29. Of the 409 plants that had flowered by that date 313 were staminate and 96 were pistillate. Only about one-third of the observed plants bloomed in 1929.

It was noticed in 1929 that the majority of plants tended to flower in definite time cycles. In 1930 the flowering cycles were more or less controlled by "cutting-back" the stalks of the plants to the top of the ground. Within three or four weeks new stalks from the same crowns showed flowers. There were three main flowering periods in 1930. These were from May 6 to June 11, July 9 to August 6, and again during late August and early September. Staminate plants were observed to flower earlier in the spring than pistillate ones, and they also flowered earlier in midsummer when all plants were "cut-back." Table 1 is the summary of the sex counts made in 1930.

TABLE 1

Summary of sex counts, 1930.

	NUMBER	PERCENT
Plants observed	2520	100.00
Plants observed in flower	2188	86.83
Staminate plants observed	1024	40.66
Pistillate plants observed	1164	46.18
Plants not observed in flower	332	13.16

From table 1 it can be seen that of the plants observed 40.66 percent were staminate, 46.18 percent were pistillate, while 13.16 percent were not

observed in bloom. In other words, of the plants that flowered there were 87.97 staminate individuals to every 100 pistillate ones.

In 1931 the 2434 plants in one plot of asparagus were observed for sex. This included the 1417 plants staked as to sex in May, 1930. Table 2 shows the results of these counts.

TABLE 2

Summary of sex counts, 1931.

	NUMBER	PERCENT
Plants observed	2434	100.00
Plants observed in flower	2243	92.16
Staminate plants observed	1149	47.21
Pistillate plants observed	1094	44.95
Plants not observed in flower	191	7.84
Of the 2243 plants flowering	..	100.00
Staminate	..	51.23
Pistillate	..	48.77

Among the 2243 plants whose sex was noted there were 105.04 staminate plants to every 100 pistillate ones.

An interesting aspect of this problem is the possibility of altering the sex ratio of asparagus through varietal hybridizations such as those mentioned in the preceding section. MALHOTRA (1930) altered the sex ratio in asparagus by aging and by manipulation of the volatile oils of its own pollen. WHITMAN (RIDDLE 1914) found that if certain very distantly related pigeons are mated that only male offspring result. If the matings were made of individuals not quite so distantly related then the first eggs produced in a season produce nearly all males, while the last eggs laid in a season produce all females.

Conclusions

In *A. officinalis* the ratio between pistillate and staminate plants is about 1:1 with considerable fluctuation for any given planting. In its approach to the 1:1 sex ratio this species conforms to a Mendelian hypothesis of sex. Staminate plants flower somewhat earlier in the spring than pistillate plants; also, when all plants are "cut-back" in midsummer staminate plants flower more quickly than pistillate.

Varietal hybridizations offer the possibility of altering the normal sex ratio.

On hermaphroditism, sex intergrades, and reversal of sex

Asparagus officinalis is the only dioecious representative among the approximately 100 species of the genus. The other species of this genus

are hermaphroditic. *A. officinalis* while functionally dioecious yet shows in the flowers of both sexes rudiments of organs of the opposite sex.

ROBBINS and JONES (1925) report the occurrence of the following flower forms in *A. officinalis:* strongly pistillate; weakly pistillate; hermaphroditic; weakly staminate; and, strongly staminate. NORTON (1913) reported the occurrence of an occasional hermaphroditic plant in his plots of this species. SHOJI and NAKAMURA (1928) in a special study on the dioecism of garden asparagus failed to note a single hermaphroditic flower in their cultures. Their studies showed that the degree of development of stunted stamens in pistillate flowers is almost the same in different individuals, while the development of pistils in staminate flowers varies very widely in different plants.

Sex intergrades occur in a great many plants. YAMPOLSKY (1920a) gives a good review of such cases reported prior to 1920, and (1920b, 1930b) reports that the different sex forms in *Mercurialis annua* can be arranged in a completely sex intergraded series from pure males to pure females.

The term "sex reversal" used in reference to unisexual plants is now usually interpreted as the occurrence of functional flowers of one sex on a plant that originally bore only flowers of the opposite sex.

PRITCHARD (1916) induced alteration of sex in both directions in *Cannabis sativa* by "mutilations." SCHAFFNER (1919, 1927), also working with hemp, reported that by other methods he obtained over 90 percent sex reversals in both directions. MCPHEE (1924) repeated the experiments of PRITCHARD and of SCHAFFNER but failed to get as large a percent of sex reversals as reported by either of the former authors. SCHAFFNER also reports sex reversals in the dioecious and supposedly dioecious species *Humulus japonicus* (1927), *Arisaema triphyllum* (1922, 1927), *Thalictrum dasycarpum* and *T. dioicum* (1927), and *Morus alba.* YAMPOLSKY (1930a, 1930b) reports the induced alteration of sex in staminate plants of *Mercurialis annua* by means of severe pruning. STRASBURGER (1910) had already performed similar experiments on this plant and had obtained alteration in both staminate and pistillate plants by means of pruning.

During 1929, 1930, and 1931 observations were made upon the sex development of 50 staminate and 50 pistillate plants marked as to sex. These plants were checked over every few days during the early part of the season, about every seven days from August 15 to September 15, and three times from September 15 until November 1. While exact counts were not made it is estimated that approximately 12,000 flowers—from the 100 marked plants—were critically examined during the three seasons. No hermaphroditic flowers were found among these and no plant was found

which showed in flowers produced at different times any apparent differences in development of the flower parts of the opposite sex.

The data on the 100 plants just mentioned were supplemented in 1930 and 1931 by observations on 1417 plants noted and labeled as to sex from May 5 to June 11, 1930. The checks (twelve in number) on these plants, while not so detailed as on the first lot of 100 plants, were critical. In 1931, a single berry was observed to start developing on a plant that was predominately staminate. This was the only case in which a flower of a hermaphroditic nature was observed in any of my plants.

While the extent of anther development in pistillate flowers and of pistil development in staminate flowers was being noted, several facts in regard to such development appeared quite pronounced. In order to ascertain whether or not appearances, in this case, were facts, the following experiments were undertaken.

Flowers from a number of staminate and also from a number of pistillate plants were dissected and examined under the 7×power of a dissecting binocular. Stamens and pistils were measured in length, and notes were taken upon style development and plumpness of the ovary.

Tables 3 and 4 summarize the results of the flowers examined.

TABLE 3

Development of sex parts in the flowers of pistillate plants.

	1930	1931
Number of plants used	26	51
Average number stalks per plant	2	1
Average number flowers examined per stalk	26	10
Pistil length	2.8 mm—3.1 mm	2.8 mm—3.2mm
Stamen length	1.5 mm	1.4—1.6 mm

Among pistillate plants the stamens in every flower on the same stalk showed approximately the same degree of development. Flowers on different stalks of the same plant also showed similar stamen development. The ovaries in pistillate flowers from the same plant appeared almost identical in size and shape. Between the flowers of different plants there were some slight variations in the size of the ovaries, but such differences seemed to form a graduating series. Such differences as were noted were not sharp enough to warrant dividing the flowers into the two groups—strongly pistillate and weakly pistillate—that ROBBINS and JONES found in California.

In table 4 five classes of staminate flowers are designated. The classes are based upon the degree of style development. They are: (1) no style;

(2) style a small stump or knob; (3) style definite but short; (4) style almost the length of those of normal functional pistils but with no trace of a stigmatic surface; (5) style approximately normal length with traces of a stigma developed upon it.

TABLE 4

Development of sex parts in the flowers of staminate plants.

CLASS OF STYLE DEVELOPMENT	NUMBER OF PLANTS	AVERAGE STAMEN LENGTH	AVERAGE PISTIL LENGTH
(a) 1930			
1	3	..	0.9 mm
2	14	..	1.1 mm
3	6	..	1.4 mm
(b) 1931			
1	20	4.1 mm	1.08 mm
2	28	4.05 mm	1.27 mm
3	9	3.7 mm	1.46 mm
4	10	3.66 mm	1.8 mm
5	4	3.8 mm	2.8 mm

It can be seen that there is considerable variation in the size of pistils in staminate flowers. Also the stamens differed slightly in size in flowers of different plants although they were always approximately the same size in flowers of the same plant. The stamens apparently were slightly larger where the pistils were smallest, and, *vice versa*, slightly smaller where the pistils were larger. Although more data are needed for a definite correlation between stamen size and pistil development in staminate flowers it appears from the studies made that five rather definite staminate flower forms appear among my plants.

These experiments substantiated the impressions obtained from field observations on the development of the organs of one sex in flowers of the opposite sex.

In this investigation attempts at artificially induced sex alteration have been exceedingly meagre but yet seem deserving of mention.

On July 24, 1929 six one year old staminate Mary Washington asparagus plants were transplanted. The treatment that was given to each is described below.

(1) To the roots of the first plant a heavy application of fresh cow manure was applied.

(2) The second plant was treated with slaked lime.

(3) The third was fertilized with sodium nitrate.

(4) The fourth plant was treated with applications of fresh cow manure, lime, and nitrate.

(5) The fifth was treated with lime and nitrate.

(6) The last plant was merely transplanted to a higher, drier, and slightly less fertile part of the field about 100 yards from its first position.

A careful check was kept on these plants until August 15, 1931. During the three seasons no stalk, branch, or single flower, gave the slightest indication of a change in sex or degree of maleness.

Careful observations were made on the results of all the "cutting-back" of plants that occurred during my investigations. It was thought that some unforeseen phenomenon might thus be noted. The stalks of over 2500 Mary Washington plants were cut to the ground twice during the summer of 1930. One row of 127 plants was "cut-back" three additional times or five times altogether. Especial attention was paid to the flowers on these 127 plants. No variations from the original flower character and no tendency toward an alteration of sex in any flower of any plant was observed in 1930.

In 1931 two plants (neither of which was previously observed in flower) were found which differed in their sex expression from the usual pure staminate and pure pistillate types. The first of these plants was fundamentally male. It developed several hundred flowers with good pollen, but also had pistils showing unusual development for staminate flowers. In one flower the pistil was developed sufficiently to become fertilized. A berry started to develop but fell from the plant before maturing. No other sets have occurred on this plant. This has been the only case of a hermaphroditic flower observed in my plots.

The other plant mentioned failed to develop functional flowers. Over 200 flowers from different stalks were observed. All of these aborted both pistil and stamens. This plant was apparently normal in its cytological behavior prior to abortion of its sex parts. Ten gemini were counted in the pollen mother cells at diakinesis.

SCHAFFNER (1922, 1927) found in *Arisaema triphyllum* that staminate plants can readily be changed to carpellate plants by keeping the full leaf surface and giving abundant water supply, and that in rich soil carpellate plants can readily be changed to staminate plants by reducing leaf surface and water supply. Drouth conditions that existed in 1930 provided natural conditions for testing this method of sex reversal on asparagus plants at the Blandy Experimental Farm. Results so far are negative with respect to reversal of sex (FLORY 1931).

Summary

A. officinalis is generally dioecious. The development of rudimentary

stamens in pistillate flowers is almost the same in different flowers of the same plant and in the flowers of different plants. There is a measurable variation in the development of pistils in staminate flowers, accompanied by variation in anther size, which suggests the occurrence of five degrees of "maleness." The last two statements are more in accord with the findings of SHOJI and NAKAMURA than with those of ROBBINS and JONES.

The rare functional hermaphroditic flowers represent the nearest approach to a natural appearing state of sex alteration that is known in this species. Meagre experiments have failed to induce artificial sex reversal in asparagus. Present indications are that it would be a difficult task to break up the normal dioecious condition in *A. officinalis* plants to any appreciable extent.

CYTOLOGICAL INVESTIGATIONS

In the investigation of *Asparagus officinalis* by SHOJI and NAKAMURA (1928) the degree of the development of pistils in staminate plants and of stamens in pistillate plants is compared, and the process of degeneration in the abortive sexual organs is traced histologically. They report 10 as the haploid number of chromosomes for the variety Conover's Colossal. KAMO (1929) and KUHN (TISCHLER 1931) also report 10 as the haploid number. LINDSAY (1930) gives a very comprehensive review of the chromosome studies that have been made on dioecious Angiosperms.

Purpose of the cytological study

(1) To study the chief features of the meiotic divisions of the microspore mother cells.

(2) To study the chief features of the meiotic divisions of the megaspore mother cells and to trace the development of the embryo sac until it is prepared for fertilization.

(3) To determine the chromosome number in the different varieties of *A. officinalis*.

(4) To observe the chromosomes for individual differences and peculiarities.

Material and methods

Cytological studies were made upon material from all the varieties at hand. By far the greater number of observations were made upon Mary Washington material. The other varieties were observed for chromosome numbers, possible differences in chromosome behavior, shape and other characteristics.

The fixatives used were Bouin's fluid, Allen's modification of Bouin's fluid, and Shaffner's chromo-acetic. Allen's modification gave the best results although Bouin's was satisfactory. The chromo-acetic solution did not give as good results as the other two.

Both staminate and pistillate buds were fixed every hour throughout the day and night. The staminate buds fixed from 12 M. to 3 A.M., especially from 1 A.M. to 2 A.M., proved to be best for a study of meiotic divisions. In the young pistillate flowers more stages in meiotic division were found to occur when fixation took place between 5 A.M. and 8 A.M.

Following fixation the buds were dehydrated, cleared in xylol, and imbedded in paraffin. Transverse and longitudinal sections were made at 5, 7, and 10 microns, and stained in Haidenhain's iron-alum haematoxylin, brazilin, and WHITE'S (1914) magdala red—safranin—and azure II. The first named stain gave the sharpest results for most work.

A limited amount of root-tip material was studied to corroborate the chromosome counts made in germinal cells. This material was secured from seeds purchased from commercial seed houses. The material was fixed in Allen's modification of Bouin's, Bouin's, and Carnoy's fluid. The first proved to be the most satisfactory. Haidenhain's iron-alum haematoxylin was used for staining in all cases.

Microsporogenesis

"Resting" microspore mother cells (figure 1) are approximately 14μ to 16μ in any dimension. These increase slightly in size just prior to the beginning of the heterotypic division. While the microspore mother cell is in the "resting" stage the nucleolus takes a much more dense stain than do the other nuclear substances.

Heterotypic division

Figure 2 represents a pollen mother cell showing increased nuclear activity. The reticulum is disappearing as the threads thicken and arrange themselves, more or less irregularly, in pairs (figures 3–4). As conjugation proceeds the pairing threads contract away from the nuclear membrane (figures 4–6) until the chromatin matter forms a dense deeply staining knot in contact with the nucleolus (figure 7). At this synizesis stage all signs of the individual threads are lost. With the utmost care in preparation, staining, and technique, it is impossible to trace the spireme thread for any distance now. As the paired threads come out of synizesis (figure 8) they thicken slightly before the open spireme (figure 9) is formed.

Careful observation reveals that in the open spireme there are always about ten main loops, that is, the number corresponding to the gametic number of chromosomes. With the utmost care in observation this statement represents a rough approximation. But upon further thickening the spireme becomes a pachynema, and the double threads become more easily identified as units.

The pairing members, some of which were rather widely diverging in the pachynema stage (figure 10), now become more or less twisted about each other (figure 11). In this condition the threads undergo a series of shortenings and thickenings (figures 12–16). This process reduces the number of times the threads are twisted about each other and eventually results in ten pairs of compact chromosomes which lie scattered haphazardly through the nucleus (figure 17). It can be clearly seen that the shortening threads are ten in number. The threads can be followed with great clearness through all the shortening and thickening process that takes place between the breaking up of the spireme and the occurrence of diakinesis. Examination of a large number of sections has failed to show the typical "second contraction" condition. Careful observation shows that in all the stages just mentioned the two individual parts of a double thread (and later—chromosome) unit are apparently not connected end to end. The evidence indicates that the mode of reduction is parasynaptic.

As the figures show, both in the later thread stages and in the diakinetic gemini six larger and four smaller pairs are consistently observable. The difference in size of these two groups while not extreme is still striking.

With the breaking up of the spireme the nucleolus grows gradually but progressively smaller until at diakinesis it is only slightly larger than the largest geminus. It has been observed to bud at all stages from leptonema to diakinesis (figures 3, 8, 11, 15, 17). In no case has a nucleolus been observed to give off more than one bud. Apparently this bud never "wanders" but always remains in contact with the mother nucleolus, even though it is sometimes entirely separated from it. After the ten pairs of chromosomes, or gemini, become well defined in diakinesis the nuclear area entirely disappears. The nucleolus then diappears and at the same time the chromosomes become slightly more condensed, and are crowded together into a compact group (figures 18–19).

As these phenomena take place a bipolar spindle becomes more and more evident and the chromosomes become arranged upon it. This spindle occurs in the area that had formerly been occupied by the nucleus, but exceeds this area and almost extends from one side of the cell to the other (figure 20). Upon casual observation the spindle appears to be quite plain,

but when individual fibers are focused upon it, it is difficult to determine their absolute point of attachment. Each fiber is apparently made up of numerous fibrils which seem to diverge slightly near the chromosomes and make the actual attachment point uncertain.

Occasionally one or two "early" chromosomes were observed to leave the nuclear plate ahead of the other chromosomes. During the heterotypic metaphase the chromosome pairs present in the equatorial plate separate, ten going to one pole, ten to the other, establishing the haploid number of chromosomes in the resulting daughter nuclei. No lagging of chromosomes was noticed.

Ten chromosomes were counted in the pollen mother cells of varieties Mary Washington, Barr's Mammoth, Conover's Colossal, Giant Emperor, Argenteuil, Palmetto, and Bonvallet's Giant. The counts were made from I anaphase plates. No morphological differences were apparent between the chromosomes of the different varieties.

Normally all the chromosomes move from the equatorial plate to the poles in approximately the same plane (figures 27–29). As the chromosomes approach the poles they condense somewhat and become more definite in shape than formerly (figures 21–26, 56).

Upon reaching the poles the chromosomes form a close circle and increase their bulk. They now show signs of having undergone an equational split. They also elongate and send out irregular pseudopodia-like projections toward the other chromosomes of the complement (figure 32). The developing daughter nuclei are enclosed by nuclear membranes; alveoli appear in the chromatin masses and do away with all traces of the individual chromosomes as the complete nucleus is formed.

In late anaphase following the reduction division a wall is formed across the spindle by thickening of the fibers at the position formerly occupied by the equatorial plate (figure 31). After the formation of the daughter nuclei the spindle all but disappears, and the wall that was first formed across the spindle is now extended to each side of the cell. Division starts at the side of the cell (figure 35), and proceeds toward the center, finally dividing the mother cell into two hemispherical cells. The daughter nuclei move to a central position in their more or less hemispherical cells, and are ready to enter the homoeotypic division phase.

Homoeotypic division

The second division takes much less time than the first. The two homoeotypic spindles are usually parallel to each other (figures 36–38). The homoeotypic spindles are usually less distinct than the heterotypic.

The univalent equationally split chromosomes become arranged on the equatorial plate (figure 36). The daughter chromosomes separate and pass toward the poles. Frequently this separation occurs earlier in one sister cell than in the other (figure 38).

Upon reaching the poles the chromosomes form dense masses. Daughter nuclei are organized and nuclear membranes enclose them. The spindles become more barrel-shaped, and often some of the spindle fibers appear heavier than formerly. As the tetrad nuclei, which have originated from a single pollen mother cell, round up, walls are formed across the spindles of the two sister cells.

Wall formation results in the production of a tetrad of uninucleated microspores (figure 39). In the majority of cases all four microspores (and at least a part of the nucleus of each) are observable in about the same plane, although occasionally the arrangement is tetrahedral. This is what would be expected from the arrangement of spindles discussed above. Each microspore develops an exine and an intine.

Corroborative chromosome counts were made in somatic cells. Twenty chromosomes were observed in the somatic tissue of the ovary of Mary Washington (figure 54) and Bonvallet's Giant material. Ten pairs of chromosomes were counted in the root-tip cells of varieties Palmetto (figure 55) and Mary Washington.

Summary of microsporogenesis

(1) The later prophase stages are very clear.

(2) The method of reduction is apparently parasynaptic.

(3) The nucleolus often buds but seemingly only once. These buds always remain in association with the parent structure.

(4) In cultivated asparagus, *A. officinalis* L., ten pairs of chromosomes were observed in the following varieties: Mary Washington, Barr's Mammoth, Giant Emperor, Conover's Colossal, Argenteuil, Palmetto, and Bonvallet's Giant.

(5) The chromosomes are more clumped in homoeotypic division than in heterotypic.

(6) The chromosomes are already split longitudinally for the second division while yet in I telophase.

(7) All trace of the individual chromosomes is lost during interkinesis.

(8) The homoeotypic spindles of sister cells usually lie parallel to each other. They are less distinct than the heterotypic spindles.

(9) In homoeotypic division the chromosomes of one cell often separate a little earlier than the chromosomes of its sister cell.

(10) Usually there is a monoplanal arrangement of the newly formed microspores.

Development of the female gametophyte

COULTER and CHAMBERLAIN (1903) regard the history of the female gametophyte as beginning with the division of the megaspore mother cell. A similar consideration is made in making the observations reported in this section. Hence in observing the development of the female gametophyte of *Asparagus officinalis* attention was paid both to the meiotic division stages and to the development of the embryo sac proper.

Normally two campylotropous ovules are borne in each of the three locules of the ovary. The method of placentation is axile. The funiculus is very short and thick and it almost appears as if the ovules were attached directly to the placentae without the intervention of a stalk.

The megaspore mother cell is developed by the enlargement of a subepidermal cell of the nucellus. Usually only one such mother cell appears in each ovule. However one case has been observed of two megaspore mother cells occurring side by side in the same ovule.

Megasporogenesis

In megasporogenesis the chromosome behavior is very similar to that observed in microsporogenesis. Hence no great detail will be gone into in the present description.

The megaspore mother cells are rather rectangular in shape, with rounded ends, and are almost oval in cross section. They are slightly thicker than the 10μ at which most sections were cut and which is quite sufficient to include the nucleus.

The prophase stages are essentially as in microsporogenesis. The reticulum disappears. Threads are formed, and pair, and contract into a synaptic knot (figure 41). On recovery from synizesis (figure 42) the chromatin threads thicken and spread out over the whole nucleus in an open spireme (figure 43). Now the threads become yet thicker and the spireme breaks up (figure 44). Following this the double threads go through numerous contractions until ten pairs of compact chromosomes result (figure 45). In such diakinesis stages the ten pairs of chromosomes are very evident.

The chromosomes seem to remain much more clumped in the I metaphase (figure 46), I anaphase, and I telophase (figure 49) stages than in the same stages of division in the pollen mother cell. However in I anaphase ten chromosomes are very evident in the complements that go to each pole (figures 47–48).

Following the heterotypic telophase (figure 49) a wall is formed between the two daughter nuclei and two secondary megasporocytes result. These two cells are arranged in an axial row within the nucellus.

The secondary megasporocytes enter almost immediately into the stages of the second meiotic division. The two homoeotypic spindles are usually arranged at right angles, or semi-right angles to each other, the one nearest the micropyle being at right angles to the axis of the nucellus. These spindles, also, are less distinct than are the heterotypic spindles.

The univalent chromosomes become arranged upon the equatorial plate (figure 50), and the daughter nuclei separate and pass toward the poles. Shortly after the chromosomes reach the poles a nuclear membrane encloses each daughter nucleus (figure 51). The camera-lucida drawings of second metaphase and telophase stages are typical.

After the four nuclei round up and are enclosed by membranes the initial stages of a cell wall form across the spindles of both sister cells. In the inner cell the wall across the spindle materializes and divides this cell into two megaspores. However the cell wall that starts to form across the spindle of the secondary megasporocyte nearest the micropyle disappears instead of extending all the way across the cell and dividing it. Thus there results from the II division three megaspores (figure 52) two of which are uninucleate, while the third one (the one nearest the micropyle) is binucleated.

Development of the embryo sac

One of the three megaspores, that nearest the chalaza, becomes functional almost immediately (figure 53). The functional megaspore enlarges and at the same time the other two megaspores become disorganized. Apparently the functional one absorbs the other two and begins its enlargement at their expense.

The inner megaspore becomes completely absorbed about the time of the first division in the embryo sac. The outer megaspore becomes disorganized as the two-celled embryo sac enlarges.

Both non-functioning megaspores are absorbed and all trace of these cells is gone by the time of the second division in the embryo sac. At this time the only indication of their previous presence is the persistence of a clear vacant space between the nuclei in the micropylar end of the sac and that portion of the megasporangium wall nearest the micropyle.

The functional megaspore increases in length from 2 to 3.5 times while its nucleus remains in the "resting" condition. The nucleus divides and a daughter nucleus passes to each end of the spore. Two more nuclear divi-

sions now occur and result in four nuclei in each end of the embryo sac. Cell formation then occurs, by which three cells, the egg apparatus, are formed in the micropylar end. This leaves the fourth nucleus free. Similarly three antipodal cells are formed in the chalazal end, leaving the fourth nucleus free, again. The free, or polar, nuclei increase considerably in size and move toward the center of the mature gametophyte. Although fertilization has not been observed many mature sacs have been examined and the polar nuclei remain, for some time at least, without uniting.

The female gametophyte is now 80μ to 85μ in length and 35μ to 40μ wide at the point of greatest width. The micropylar end is much larger than the chalazal end. The latter end is often curved slightly away from the main axis of the gametophyte, and is almost filled by the three antipodal cells. Very little cytoplasm appears in the mature embryo sac except that included in the cells at either end.

Individuality of the chromosomes

Recent observations on dioecious Angiosperms have revealed (LINDSAY 1930) that in the majority of these plants investigated there is a difference in one sex or the other between one or more pairs of the chromosome complement. In these cases the behavior of the chromosomes is shown to be correlated with the expression of the sexes. It seems only logical therefore that in a cytological study of the present dioecious species especial attention should be given to the peculiarities of the several chromosomes.

Observations were made on the chromosomes of *A. officinalis* at all stages during the meiotic divisions. At most stages from shortly after the breaking up of the spireme in I prophase (figures 13–14) until II anaphase (figure 37) ten chromosomes or chromosome pairs can be observed. Of the ten chromosomes six are always noticeably larger than the other four. Although this difference is slight it is very constant. The four smaller chromosomes (figure 56, VII–X) are rather cylindrical in shape. Roughly, when viewed from the side, they are from 2 to 3 times as long as thick. When a polar view of them is observed they appear roughly circular. These four chromosomes form an almost imperceptibly graded series in size. The six larger chromosomes (figure 56, I–VI) are more angular in shape. A side view of them offers the greatest visible area and hence gives a truer idea of their general shape. When viewed from different angles these chromosomes appear much different in shape. At certain angles several of them appear to be no larger than the largest of the four smaller chromosomes. Careful focusing with 3000$\times$ magnification reveals the difference.

Certain individual differences are also apparent among the chromosomes of any given figure. The chromosomes are more condensed in size and slightly more definite in outline during I anaphase than at any other stage. Many anaphase chromosome plates were studied with particular attention being directed toward any individual peculiarities of the chromosomes. In figure 56, A to N represent the chromosomes from fourteen typical anaphase plates drawn with an Abbe camera-lucida at a magnification of 5150×. The chromosomes from these fourteen figures were matched as nearly as possible in size and shape. The result of this matching is seen in columns I to X. From the study of anaphase plates and the corroboration of this study by observations on the chromosomes in other stages of division it is seen that during meiosis the chromosomes present some quite individualistic size and shape characters. These are described in detail in an earlier paper (FLORY 1931).

Many homologous haploid chromosome complements were examined at I anaphase stages. These emphasized the essential similarity of daughter chromosomes. Sometimes the homologous complements were in the same section and either group of chromosomes appeared in a striking way by focusing up or down. Figures 24 and 25, as well as figures 22 and 26, are haploid complements passing to opposite poles of the same spindle. The daughter chromosomes are seen occupying the same respective position upon the spindles. In figure 56, F and G, and I and J, are homologous chromosome groups.

Study of the anaphase figures in the dividing megaspore mother cell shows that here also six of the ten chromosomes are larger than the other four (figures 47–48). The same general peculiarities of the separate chromosomes occur here that were previously noted for the chromosomes of pollen mother cells.

Somatic chromosomes were studied in several cases. In the somatic tissue of the ovary (figure 54) and in root-tip material (figure 55) twenty chromosomes appear in the nuclear plates. Ten of these appear to be duplicates of the other ten. No differences are noted between the members of any pair of chromosomes.

In the somatic figures also, four pairs of chromosomes are smaller than the other six pairs. This is more evident in root-tip material than elsewhere. Chromosomes in somatic figures lack the angularity which gives the meiotic chromosomes their distinctive shapes but yet show differences that are at least suggestive of parallel peculiarities.

DISCUSSION ON THE MODE OF SEX DETERMINATION

Much experimental work with sex in plants and animals has given rise

to two rather well-defined schools of thought concerning the manner in which sex is determined. They are the genic and the physiological schools respectively. A survey of the literature dealing with sex studies in the phanerogamic plants shows that in this group of organisms alone there is a mass of evidence in favor of the two theories.

Four different sources of evidence from the dioecious seed plants favor the theory that in this group sex is determined according to a genic, or a Mendelian, hypothesis. These sources of evidence are: (1) hybridization experiments performed by CORRENS (1907), BATESON (1913), SHULL (1910, 1911) and many others; (2) sex-linked inheritance demonstrated by BAUR (1912), SHULL (1914), and WINGE (1927, 1931); (3) sex ratios which usually conform to the I:I proportion expected with the genic hypothesis; and (4) cytological observations which have revealed that 43 out of 66 species of dioecious Angiosperms studied cytologically show a chromosome difference between the staminate and pistillate plants (LINDSAY 1930).

Evidence advanced for the physiological theory of sex determination has, for the most part, had its origin in observations on the effect of different environments on alteration in expression of sexual characters. Several outstanding examples of the complete reversal of sex have been reported from the animal world by CHAMPY (1921), by CREW (1923), and by RIDDLE (1914, 1925). SCHAFFNER (1919–1931) and YAMPOLSKY (1920a, 1920b, 1930a, 1930b) have been outstanding in attributing to physiological conditions the determination of sexual expression in dioecious seed plants.

Up to the present time the culmination of the theories of sex determination in the dioecious seed plants, as well as in all the higher plants, is realized in CORRENS' (1928) work setting forth his empirical formula for sex determination and inheritance. CORRENS, in his theory, conceives of four general types of sex. The dioecious seed plants he groups under type IV. By this theory the sex of the members of type IV is genotypically determined, the following genes or gene complexes being involved in the determination: (1) G—is responsible for the formation of the gynoecium; (2) A—is responsible for the formation of the androecium; (3) Z—determines the time and place activities of the uniformly acting genes A and G; and (4) the "Realizators": α' affecting the gene complex A, and γ' affecting the gene complex G. The "Realizators" may have weak or strong valencies and may play the role of accelerators or of inhibitors. In plants in which the staminate are heterozygous for sex (and present experimental data indicate that this is true of the majority of the dioecious seed plants) the diploid sex formulae would be AAGGZZ$\gamma'\gamma'$ ♀ and AAGGZZ$\alpha'\gamma'$ ♂. In

cases where the pistillate plant is heterozygous for sex the diploid sex formulae would be AAGGZZ$\alpha'\gamma'$ ♀ and AAGGZZ$\alpha'\alpha'$ ♂.

Under this formula the environment expresses its influence on the gene or gene complex Z, and this in turn activates A or G, affecting their states of being, functional or otherwise. Hence CORRENS has incorporated both the genetic and the metabolic theories of sex determination into one formula and has put the whole matter on a factorial or Mendelian basis.

In the monoecious flowering plants grouped under type III, Z determines the location and often the sequence of the development of A and G. Hence in the monoecious flowering plants the development of A may occur at an earlier, or an entirely different, period than the development of G, or *vice versa*. This actually does occur in a number of cases in monoecious plants. There is danger of confusing these plants with the dioecious plants grouped in type IV. In dioecious plants sex is determined genotypically by the "Realizators"; in the case of monoecious plants the sex of the inflorescences is phenotypically determined, annually, biennially, seasonally, etc. According to CORRENS such monoecious plant conditions have been confused with dioecious plant conditions by SCHAFFNER and by MAEKAWA working on Arisaema. Such is most probably the case with YAMPOLSKY working on Mercurialis. When this point is clearly seen some of the most important evidence for a purely physiological theory of sex determination in the higher plants is rendered untenable. A more complete discussion of this has been given in an earlier paper (FLORY 1931).

On the basis of present data the determination of sex in any dioecious seed plant can be explained on a genic basis by means of CORRENS' formula, provided that the sex condition of a plant has been carefully analyzed and that it really belongs in CORRENS' group IV.

A. officinalis will now be examined from the standpoint of its mode of sex determination. The observations on this form—both original and reported—will be considered. From the light thrown on the determination of sex in dioecious plants as a whole the present task should not be a long one. The data available all support CORRENS' theory as expressed in his empirical formula of sex determination in the higher plants. Because of this it seems the feasible thing to see where and how *Asparagus officinalis* fits into CORRENS' theory.

A. officinalis apparently belongs in type IV. The plants are nearly always entirely staminate or entirely pistillate. The ratio of staminate to pistillate is approximately 1:1. This fact is best explained on the assumption that one sex is heterozygous for sex and such an assumption agrees with one of the fundamental hypotheses by which CORRENS explains the

determination of sex in the members of this group. No cases have ever been reported of the appearance of pistillate flowers on normally staminate plants or of staminate flowers on normally pistillate plants. *A. officinalis* does deviate from the true type dioecious seed plant in these several ways: (1) in the flowers of each sex the rudiments of the sex organs of the opposite sex are present; and (2) the occasional occurrence of hermaphroditic flowers upon male plants due to the development of the usually rudimentary pistil to a point where it is capable of functioning.

CORRENS provides for plants with just such conditions in type IV of his theory. In this group besides the purely dioecious plants there are also grouped other dioecious plants in which "there appear in the same individual besides flowers of the one sex also some hermaphroditic flowers or flowers of the other sex." Plants of this latter kind CORRENS designates "subdioecious" after DARWIN (1877). CORRENS explains the occurrence of subdioeciousness thus: "Differences in the completeness of the sexual separation are easily understood when one thinks of the often repeated independent phylogenetic origin of the 'mixed sex' condition. *The less sharp separation rests on a less strong effect (weaker valency) of the later acquired realizators in contrast to the originally received anlagen complex remaining present which conditions the 'sex-mixed' condition of the original form,* derived from the separate sexed forms. The 'mixed-sex' tendency becomes suppressed now completely and now more or less far-reachingly, according to the species and, as one can also say, according to external conditions. . . . One can call this behavior, with Darwin, 'subdioeciousness' when it is the rule and not the exception." Examples that CORRENS gives of subdioecious plants are *Silene roemeri, Spinacia, Humulus,* etc.

From this and from the descriptions that have already been given of the structure of the *A. officinalis* flower there can be no doubt that under CORRENS' theory this species would be classed as "subdioecious" and grouped with the plants included in his type IV.

Now that the species is placed as to type there is yet to be decided which sex is heterozygous for that character. Evidence from hybridization is lacking along this line. Also a cytological study has divulged no hint of either sex being heterozygous, although a pair, or pairs of chromosomes may presumably differ physiologically because of their genes without this difference being made apparent in a "phenotypic" expression of the chromosomes. But notwithstanding the lack of evidence from these sources it seems very apparent that the staminate plants are heterozygous for sex, and the pistillate plants are homozygous for sex. The rare appearance of hermaphroditic flowers is always on staminate plants. Such flowers have

never been reported to appear on pistillate plants. Then, too, the development of the rudimentary stamens in pistillate flowers always proceeds to approximately the same extent before abortion. On the other hand considerable variation is observed in the development of the rudimentary pistils in staminate flowers. Such pistils vary in size from small "nubbin-like" ovaries without styles to, in rare cases, the weakly functional pistils present in hermaphroditic flowers. These facts are quite indicative of the heterozygousness of the staminate sex, and the homozygousness of the pistillate sex.

Now if the sex situation in *Asparagus officinalis* is described by CORRENS' theory of sex determination expressed in the formula terms explained above, for normal cases the pistillate diploid formula for sex would be AAGGZZ$\gamma'\gamma'$, while the staminate diploid formula would be AAGGZZ$\alpha'\gamma'$.

A. officinalis is subdioecious because the "Realizators" do not become effective until later in the development of the plant than is true in the case of strictly dioecious plants. At first only the complexes A, G, and Z affect the development of sex in the plants of this species. Apparently the "Realizators" do not affect the sexual expression of a plant until some time after the flower itself is developing. In other words this is a subdioecious plant in which at first both A and G are present without either the accelerating or inhibiting effect of "Realizators." Under normal conditions Z has the same effect on both A and G. The result is that when a flower starts to develop it has an equal impetus for the production of both pistillate and staminate sex organs. At a certain stage in the growth of the flower, which from the results is seen to be a rather constant point, the effect of the "Realizators" comes into play. In plants which contain the $\gamma'\gamma'$ complex a strong inhibiting influence is directed on A which overcomes the activating tendencies of Z toward this complex and results in the suppression of further growth of the staminate flower parts by the accelerating impetus which is given to the G complex and which results in the maturity of the gynoecium. In plants, which contain the $\alpha'\gamma'$ complex, α' has much the stronger valency of the two. It accelerates A which results in the maturity of the androecium. The weaker valency of γ' often results in a complete suppression of G, and the pistil, which up to this time has been developing as rapidly as the stamens, ceases growth and never becomes more than a tiny "nubbin-like" ovary with a faint suspicion of a style. But by the terms of the theory the "Realizators" may have weak or strong valencies. Hence sometimes α' is not quite so strong and γ' has a slightly stronger valency. Then, through the effect of these factors on A and G, a slightly

better developed ovary results; and, very rarely, γ' is strong enough in relative valency to develop, through G, a functional, but a rather weak and incomplete, pistil. With a stronger valency of γ' there occurs a proportionately weaker valency of α'. Under such conditions the accelerating influence of α' on A is lessened, and the stamens, although plump and entirely functional, are not quite as large as in other cases and form a graduated size series varying proportionately with the valency strength of α'.

Normally after the appearance of the expression of the "Realizators," the effect of α' and γ' on A and G rather obscures the action of Z on A and G. However there exists the chance that under abnormal circumstances, with the proper nutrition, or with a shortened light period, or with other changes in environmental conditions, the environment would cause Z to have a stronger action on A or G, or both, than is the case ordinarily. Indeed it can readily be imagined that under certain definite conditions Z would so activate A or G, or both, that reversal of sex would appear in either or both directions, from the staminate to the pistillate, or from the pistillate to the staminate.

Hence the determination of sex in *Asparagus officinalis* can be explained, with the aid of CORRENS' formula, on a genic basis which considers all the factors, internal or external, that may influence sexual expression.

GENERAL SUMMARY

Asparagus officinalis L. is a subdioecious plant species in which staminate and pistillate individuals occur in approximately a 1:1 ratio. The evidence indicates that the staminate plants of the species are heterozygous for sex, since only these bear the rare hermaphroditic flowers. The mode of sex determination may be explained by CORRENS' empirical formula.

The rarity of varietal differences, the few vegetative differences apparent between the members of the two sexes, the rare occurrence of sex intergrades, the absence of naturally occurring sex reversal, and the regularity of the chromosome behavior are facts indicative of the comparative non-variability of this species.

The behavior of the chromosomes during microsporogenesis and megasporogenesis is described.

The haploid number of chromosomes is 10 in *Asparagus officinalis* varieties Mary Washington, Barr's Mammoth, Conover's Colossal, Giant Emperor, Argenteuil, Palmetto, and Bonvallet's Giant. The diploid number was observed to be 20 in varieties Mary Washington, Palmetto, and Bonvallet's Giant.

Six members of the ten pairs of chromosomes are larger than the other four. The several chromosomes evince individual peculiarities both in a haploid and in a diploid condition. There are no apparent differences between the two members of any pair of chromosomes.

The method of reduction of the chromosomes is parasynaptic.

The megaspore mother cell develops into three megaspores, two uninucleate, the other binucleate. One of the uninucleate megaspores develops into an eight nucleated embryo sac, and the other two degenerate.

The most favorable time for obtaining division figures in microspore mother cells is from 12 M to 3 A.M.

The writer wishes to thank Doctor ORLAND E. WHITE for the advice, suggestions, and criticisms he offered during the course of these investigations.

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Explanation of Plates

The drawings were made with a Zeiss microscope equipped with a 2 mm apochromatic objective, a 1.8 mm achromatic objective, and 20× and 30× compensating oculars. All drawings were made at table level with the aid of an Abbe camera-lucida. The magnification of figures 54, 55, and 56 is about 5150×. In the remainder of the figures the magnification is about 3200×. In reproduction plates 1, 2, and 3 were reduced one-third; plate 4 was reduced one-fifth. Unless otherwise noted the figures are drawings of variety Mary Washington. Figures 1–39 depict microsporogenesis; figures 40–53 deal with the development of the female gametophyte.

Legend for Plate 1

Figure 1.—"Resting" microspore mother cell.

Figure 2.—Early prophase stage.

Figures 3–5.—Zygonema stages. Pairing of the threads is evident.

Figure 6.—Early synizesis.

Figure 7.—Synizesis.

Figure 8.—Recovery from synizesis.

Figure 9.—Open spireme.

Figure 10.—Pachynema stage.

Figure 11.—Pachynema stage. The nucleolus is budding.

Figure 12.—Diplonema stage. Six pairs of threads are visible.

Figure 13.—Strepsinema stage.

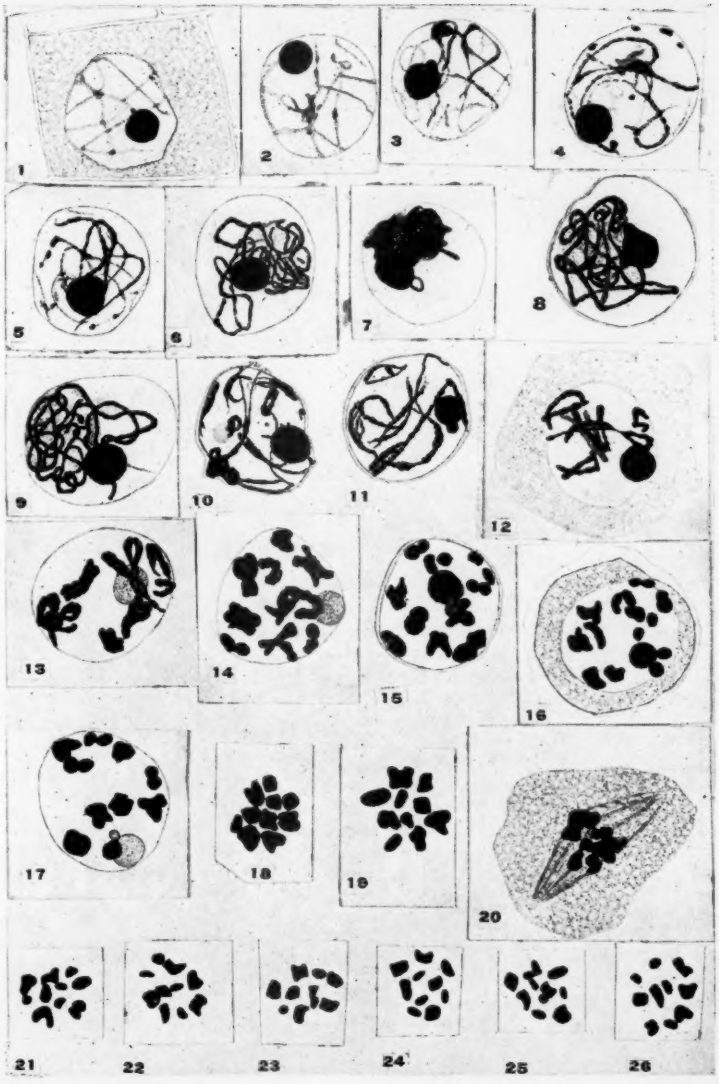
Figures 14–15.—Chromosome pairs shortening and thickening.

Figures 16–17.—Diakinesis. Ten gemini are present, six larger than the other four. The nucleoli are somewhat smaller than formerly.

Figures 18–19. Polar views of ten bivalent chromosomes on the equatorial plate. I metaphase stage.

Figure 20.—Lateral view of the same stage.

Figures 21–26.—Polar views of heterotypic anaphases.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LEGEND FOR PLATE 2

FIGURE 27.—Polar view of very early heterotypic anaphase.

FIGURE 28.—Polar view of early heterotypic anaphase (Conover's Colossal).

FIGURE 29.—Lateral view of heterotypic anaphase.

FIGURE 30.—Lateral view of abnormal late heterotypic anaphase.

FIGURE 31.—Lateral view of heterotypic telophase.

FIGURE 32.—Polar view of late telophase.

FIGURES 33–34.—Polar view of earlier telophase figures.

FIGURE 35.—View of the pollen mother cell at interkinesis with cell division taking place by cell wall formation.

FIGURE 36.—Lateral view of homoeotypic metaphase.

FIGURES 37–38.—Polar and lateral views of homoeotypic anaphase.

FIGURE 39.—A tetrad of newly formed microspores, monoplanal arrangement.

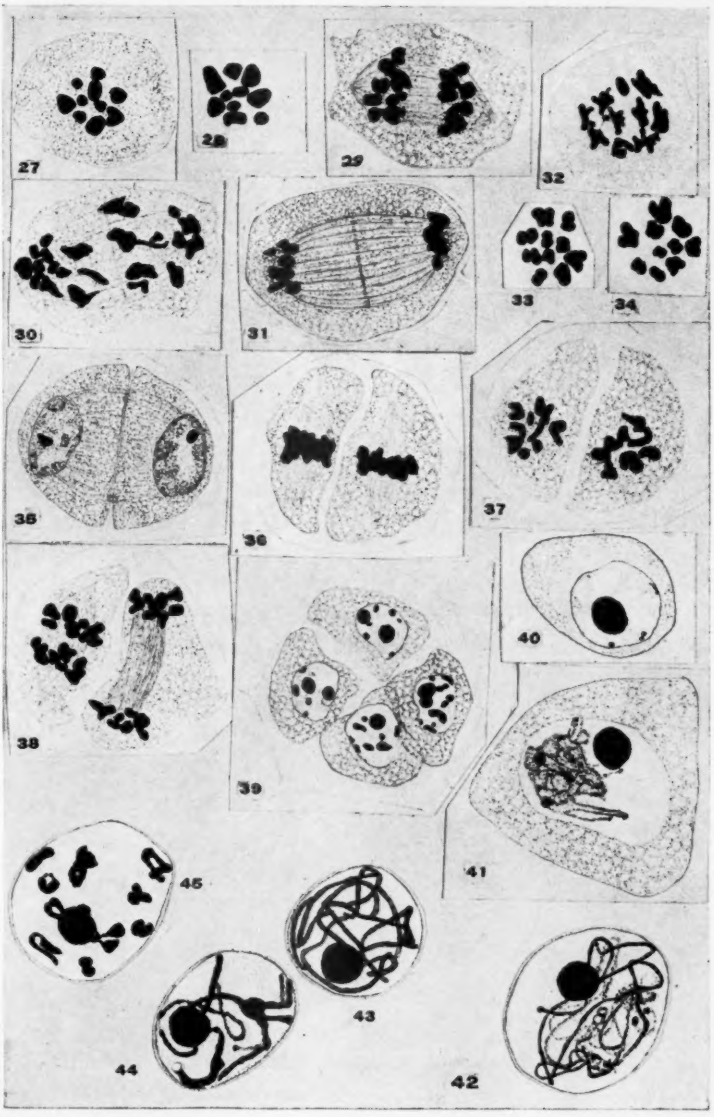
FIGURE 40.—"Resting" megaspore mother cell.

FIGURES 41–42.—Synizesis and recovery from synizesis, respectively.

FIGURE 43.—Open spireme stage.

FIGURE 44.—Early pachynema stage.

FIGURE 45.—Early diakinesis stage.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LEGEND FOR PLATE 3

FIGURE 46.—Lateral view of heterotypic metaphase.

FIGURES 47–48.—Heterotypic anaphase, polar views.

FIGURE 49.—Lateral view of heterotypic telophase.

In the four following figures the micropylar ends are directed toward the top of the plate.

FIGURE 50.—Homoeotypic metaphase, lateral view.

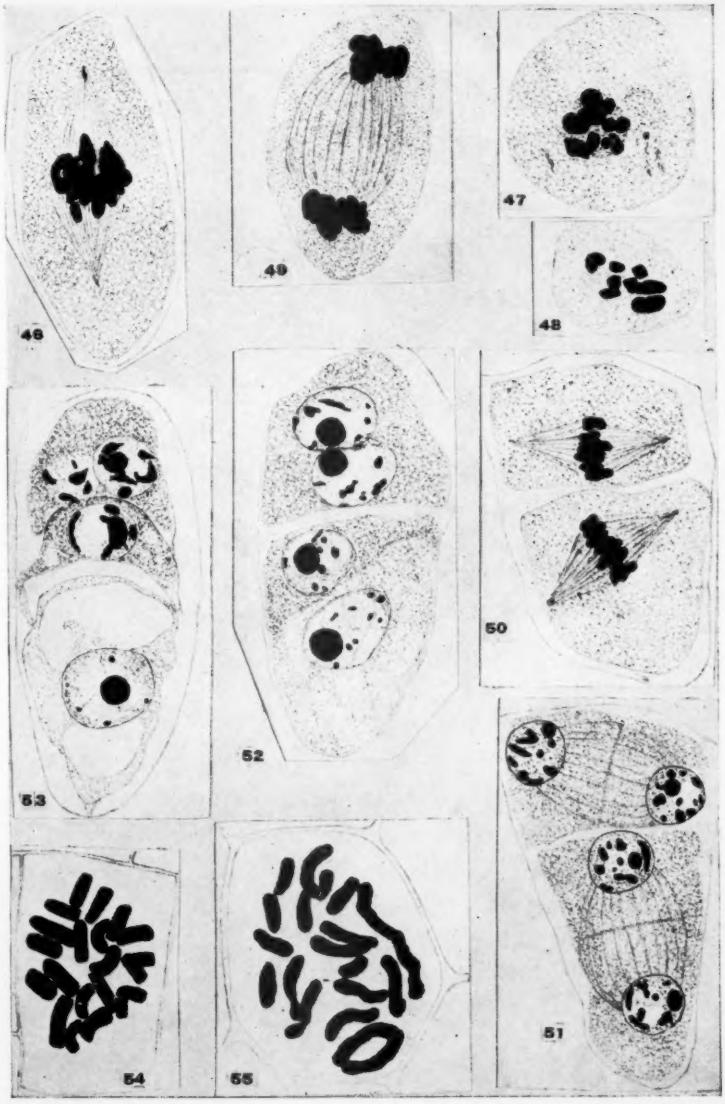
FIGURE 51.—Homoeotypic telophase.

FIGURE 52.—Three megaspores. The spore nearer the micropyle is binucleated, the two nearer the chalaza are uninucleated and crowded very close together. The inner megaspore is beginning to develop.

FIGURE 53.—The megaspore nearer the chalaza is enlarging and becoming vacuolar at the expense of the micropylar ones.

FIGURE 54.—A somatic cell from the ovary showing 20 chromosomes.

FIGURE 55.—Twenty chromosomes in a root-tip cell of Palmetto material.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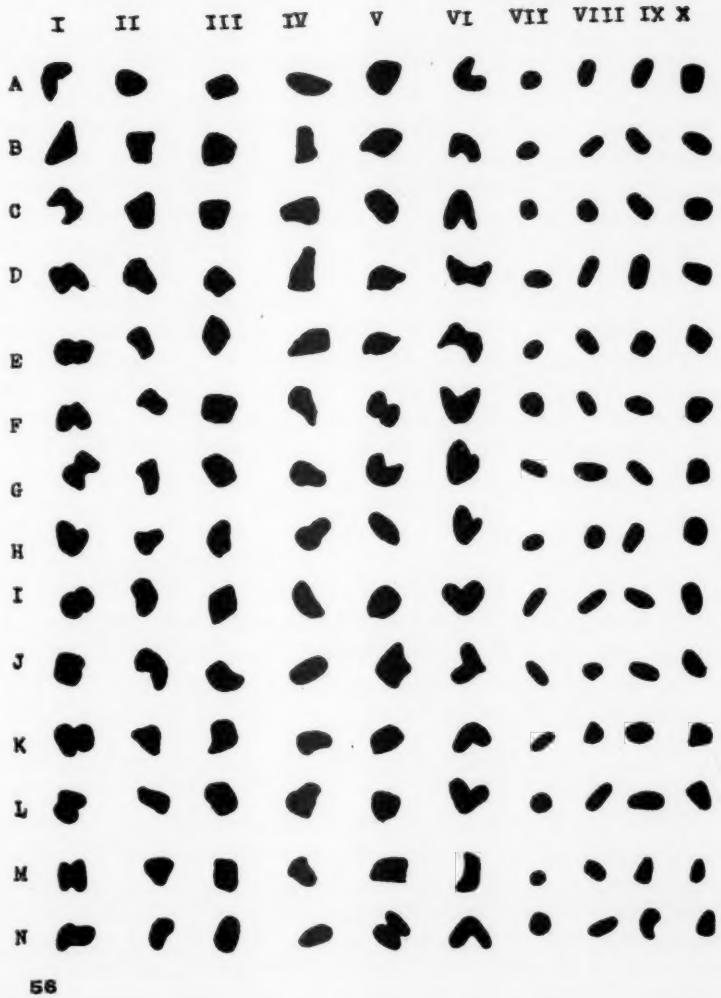
LEGEND FOR PLATE 4

FIGURE 56.—Fourteen heterotypic anaphase figures with the chromosomes drawn individually at a higher magnification than was used in most of the other figures. Vertically each row (A-N) represents a different anaphase figure. Horizontally the columns (I-X) represent the ten chromosome types found in each haploid complement.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A GENETIC ANALYSIS OF DISSIMILAR CARCINOMATA FROM THE SAME GLAND OF AN INDIVIDUAL MOUSE

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A comparative study of the transplantability of eight spontaneous mammary gland tumors was recently made by the author (1932). These tumors occurred in the old breeding females of a race of inbred mice. Three of the tumors developed independently of each other in different regions of the mammary gland of a single individual. In the course of the experiment it became evident that regardless of the similarity in origin and histology of these three tumors, no two of them were alike in all their responses to transplantation. This dissimilarity was detected while employing the individuals of the segregating hybrid generations from a cross of a susceptible with a non-susceptible race of mice.

The question naturally arose: Can the same gland of an individual give rise simultaneously to genetically and physiologically different tumors which are morphologically alike? This paper is an attempt to answer this query.

The animals used were from different inbred stocks of *Mus musculus* and their hybrids. The pure stocks have all been maintained for years by strict brother to sister matings. They were intensively inbred previous to the start of the experiment in 1928. The tumors investigated arose in an albino female of the twenty-sixth generation of brother to sister matings. This race is homozygous for albinism, non-agouti, intensity, brown and self. It is STRONG'S derivative of the Bagg albino strain and is called the A stock. These mice are susceptible to transplants of all investigated tumors from other A stock mice. The dilute brown mice, or D stock, were used as a non-susceptible race of individuals. These are STRONG'S sub-line of LITTLE'S stock and are homozygous for color, non-agouti, dilution, brown, and self. In addition, a dilute brown piebald race, the N stock, was secured from STRONG as a stock the individuals of which were non-susceptible to tumor 13738b. These mice are homozygous for color, non-agouti, dilution, brown and piebald. None of the above stocks are related. (For additional information on these races, see BITTNER [1929] and CLOUDMAN [1932].)

Members of the susceptible A stock were crossed with those of the non-susceptible D stock and the first and second filial generations and the backcrosses to mice of both parent types were secured. Only individuals

of the F_1 and F_2 generations were employed from the cross between mice of the A stock with those of the N stock.

Female S 13738, of the A stock, was killed at fourteen and one-half months of age and three tumors (a, b, and c) were removed from her. These were used for the transplantation experiment. Tumor 13738a occurred just anterior to the right iliac region, 13738b occurred in the left-axillary region, and 13738c occurred in the left iliac region. All three were diagnosed as carcinomata of the mammary gland.

The interpretation of this work is based upon the multiple factor hypothesis of LITTLE (1914 and 1920), LITTLE and TYZZER (1916), and LITTLE and STRONG (1924). This means that susceptibility to transplanted tumors depends upon the simultaneous presence, in the host, of dominant multiple Mendelian factors coming from the susceptible parent stock. When we cross mice of a susceptible with those of a non-susceptible stock, all the F_1 individuals are susceptible. This shows that the heterozygous condition of all the susceptibility factors is sufficient to allow the growth of the transplanted tumor. The mice of the (+BC) generation, or backcross to the susceptible parent, are all susceptible while the (−BC) mice, or backcross to the non-susceptible parent, and the mice of the F_2 generation show a segregation of susceptibility giving definite ratios of susceptible to non-susceptible animals. Thus the number of susceptibility factors involved is detected from the data for the F_2 and (−BC) generation individuals when large numbers of animals are employed.

RESULTS

The reactions of these three tumors in individuals of A stock, D stock, and their hybrid generations are shown in table 1. Male Cl 1769 of the A stock was negative to tumors 13738a and 13738b. He was a product of thirty-three generations of unbroken brother to sister matings and had two susceptible brothers. This gives but one exceptional A stock animal out of 631 who received transplants of these tumors. There were no exceptional F_1 (D×A) individuals, for all grew the tumor tissue which they received. One (+BC) female B 2462 failed to grow tumor 13738b even after receiving a second transplant. This gave a difference of 00.92 percent between the observed and expected results which is probably not significant.

The D stock individuals should, theoretically, be homozygous for all factors not lethals or linked with lethals, since they have been intensively inbred since 1909. They should all respond alike to all implants of any one tumor. This is exactly what happened with implanted tissue from tumors 13738a and 13738c where all the mice employed failed to grow these tu-

TABLE 1

Reactions of individuals of A stock, D stock, and their hybrids to each of the three transplanted tumors from females S13738 (susceptible=[+], non-susceptible=[−]).

3 TUMORS FROM ♀ S 13738	INDIVIDUALS OF PURE STOCKS						INDIVIDUALS OF (D×A) GENERATIONS											
	A STOCK REACTIONS			D STOCK REACTIONS			F_1 (D×A) REACTIONS			(+BC) REACTIONS			F_2 (D×A) REACTIONS			(−BC) REACTIONS		
	+	−	Total	+	−	Total	+	−	Total	+	−	Total	+	−	Total	+	−	Total
13738a	210	1*	121	0	96	96	106	0	106	103	0	103	150	144	294	52	153	205
13738b	264	1*	265	22	48	70	135	0	135	107	1	108	206	102	308	64	135	199
13738c	155	0	155	0	75	75	121	0	121	87	0	87	190	171	361	36	150	186
Totals			631			241			362			298			963			590
Exceptional Individuals	1(♂Cl 1769)*			22 with b			0			1								

mors. The unexpected susceptibility of 31.43 percent of the D stock individuals to transplants of tumor 13738b (see table 2) showed that this tumor was actually different from the other two. Several of the D stock animals which grew 13738b received implants of the other two tumors but failed to grow them. When we analyze the observed ratio of the data for tumor 13738b in the D individuals we find it differs very significantly from the ratio expected for a stock all of whose members are homozygous for the recessive allelomorphs of the factors for susceptibility.

TABLE 2

Reactions between tumor 13738b and the D stock individuals employed.

OBSERVED		EXPECTED		DEVIATION PERCENT	DEVIATION P.E.	PROBABLE GROWTH FACTORS
REACTION	PERCENT NEGATIVE	FACTORS	PERCENT NEGATIVE			
13738b in D 70 mice		Pure Stock	100	31.43	..	..
22+:48−± 2.61	68.57±3.73	1 recessive	75.00±3.48	6.43±5.10	1.26	1 recessive
		2 recessive	93.75±1.95	21.18±4.20	5.99	

Tumor 13738b was transplanted into members of the N stock but all these individuals employed were non-susceptible to this tumor.

As already stated the segregating hybrid generations should show the number of Mendelian factors involved in susceptibility to each of these transplanted tumors. The results obtained when F_2 and (−BC) individuals were employed to receive implants of these three tumors are shown in tables 1 and 3. The observed ratio for tumor 13738a (table 3) does not differ significantly from the ratio expected when two Mendelian factors are

TABLE 3

Number of genetic factors probably needed for the successful growth of transplants of each of the 3 tumors from female S 13738.

OBSERVED		EXPECTED		DEVIATION PERCENT	DEVIATION P.E.	PROBABLE GROWTH FACTORS
REACTION	PERCENT NEGATIVE	FACTORS	PERCENT NEGATIVE			
13738a in F_2		1	25.00±1.70	23.97±2.60	9.21	
(D×A)294 mice		2	43.75±1.95	5.22±2.77	1.88	2
150+:144−						
±5.78	48.97±1.97	3	57.81±1.94	8.84±2.76	3.20	
13738a in (−BC)		1	50.00±2.35	24.63±3.11	7.91	
205 mice		2	75.00±2.03	0.37±2.88	0.012	2
52+:153−						
±4.20	74.63±2.05	3	87.50±1.55	12.87±2.57	5.02	
13738c in F_2		1	25.00±1.53	22.36±2.33	9.59	
(D×A) 361 mice		2	43.75±1.75	3.61±2.48	1.45	2
190+:171−						
±6.39	47.36±1.77	3	57.81±1.75	10.45±2.48	4.21	
13738c in (−BC)		1	50.00±2.46	30.63±3.13	9.78	
186 mice		2	75.00±2.13	5.64±2.88	1.95	
		3	87.50±1.63	6.86±2.53	2.71	2(or 3)
36+:150−						
±3.63	80.64±1.94	4	93.75±1.19	13.11±2.27	5.77	
13738b in F_2		1	25.00±1.66	8.11±2.45	3.31	
(D×A) 308 mice		2	43.75±1.90	10.64±2.62	4.06	2 when corrected
206+:102−						
±5.56	33.11±1.81					
*196.45+:111.55−						
±5.68	36.21±1.85	2	43.75±1.90	7.54±2.65	2.84	
13738b in F_2		1	25.00±1.86	16.46±2.81	5.85	
(N×A) 246 mice		2	43.75±2.12	2.29±2.99	0.76	2
144+:102−						
±5.20	41.46±2.11	3	57.81±2.12	16.35±2.99	5.46	
13738b in (−BC)		1	50.00±2.38	17.83±3.26	5.45	
199 mice		2	75.00±2.06	7.17±3.05	2.35	2
64+:135−						
±4.44	67.83±2.24	3	87.50±1.59	19.67±2.74	7.17	
*46.55+:152.45						
−±4.02	76.60±2.02	2	75.00±2.06	1.60±2.88	0.55	

* Ratios are corrected for excess susceptible individuals.

involved in the growth of this tumor. This is true for the data obtained with individuals of both the F_2 and (−BC) generations. In the latter case there is practically no difference between the expected and the observed ratios. Furthermore the ratios expected for one and for three growth factors are significantly different from the observed ratios for both generations. It is, therefore, quite probable that only two Mendelian factors for susceptibility were needed for the progressive growth of tumor 13738a in those animals which were susceptible. In the case of tumor 13738c the data for the F_2 generation individuals show that the need of one and three Mendelian factors for the growth of the transplant is definitely excluded, while the expected ratio when two factors are involved does not differ significantly from the observed ratio. The data obtained with the (−BC) individuals definitely exclude the possibility that less than two or more than three susceptibility factors are needed but the possibility that three factors are needed is not excluded. The difference between the observed and expected ratios in this latter case is, however, 2.71 times the probable error which is approaching significance. The ratio expected when two factors are needed is much nearer to the observed ratio. We can say that susceptibility to tumor 13738c is probably due to two (or possibly three) dominant Mendelian factors (table 3). We have seen that the growth of tumors 13738a and 13738c is in each case probably dependent upon the presence of two Mendelian susceptibility factors. The susceptible animals have received them from the parent mice of the A stock. The identity of these factors is taken up later on in this paper. These results are entirely confirmatory of the working hypothesis explained above.

When we examine the data for the third tumor from female S 13738 we find a different response to transplantation in the segregating hybrid individuals. By scanning the total data for the reaction of tumor 13738b implanted into the individuals of the F_2 (D×A) generation we find that the observed ratio differs significantly from the ratios expected if the presence of either one or two susceptibility factors are involved. The observed ratio lies between these two expected ratios. Analyzing the (−BC) data shows that the observed ratio might be explained if two susceptibility factors were needed (table 3). When arranged by individual experiments in chronological order the observed ratios for these two hybrid generations are in both cases apparent fluctuations of the same ratios. A division of the data into early and late periods of experimentation is not warranted. For this reason we cannot assume that a somatic mutation (STRONG) has occurred in the tumor. Furthermore contemporary experiments with tumor 13738b implanted into the F_2 (N×A) mice showed no evidence that such a change

had taken place. On the contrary the observed ratio for the reaction of this tumor in the F_2 (N×A) individuals differed by only 0.76 times the probable error from the ratio expected when two susceptibility factors are needed for the successful growth of this tumor (table 3). The observed ratio in this case also differs very significantly from the ratios expected should one or three factors be needed.

This shows clearly that tumor 13738b could grow successfully in the presence of two susceptibility factors brought into the N×A cross by mice of the A stock. It is also probable that the members of the A stock contributed these same two susceptibility factors to the hybrids of the D×A cross. With this fact in view let us return to table 2 where we find that:

(1) Certain D stock individuals, but not all, have a tendency (probably genetic) to encourage the growth of tumor 13738b.
(2) The data of the reactions of the D individuals are not significantly different from a 3− :1+, or a one factor Mendelian ratio.
(3) Susceptibility in the D individuals appears like the homozygous recessive condition (*ii*) of a dominant inhibitor allowing the growth of implants of 13738b.
(4) This "*ii*" factor combination apparently operates independently of the dominant susceptibility factors carried by the A stock mice.

In view of the data in tables 2 and 3 we know that:

(1) This 3:1 ratio could remain in this proportion indefinitely, for in the absence of selection, an equal number of *I* and *i* gametes would be formed in the population as a whole.
(2) A cross of A mice, homozygous for all the dominant factors necessary for the growth of tumor 13738b, with D mice, whose members give a 3− :1+ ratio of susceptibility to this tumor, should show distorted ratios of susceptibility in the segregating hybrid generations. Just such ratios were secured with 13738b (table 3).

In an attempt to analyze the reactions of tumor 13738b several hypotheses have been tried but only one of them is given here as a possible explanation. It fits the observed data fairly closely but it is only a working hypothesis. By this hypothesis the D stock is composed of three types of individuals in the proportion of approximately 1 *II*:2 *Ii*:1 *ii* or three non-susceptible animals with the dominant inhibitor to one susceptible animal with the *ii* factor combination. The distorted ratios obtained in the F_2 and (−BC) generations of a D×A cross would be due to excess susceptible individuals caused by this distribution. A certain number of the hybrids, like some of the D individuals, would be susceptible because of the presence of the *ii* factor. This susceptibility should show itself without the aid

of the susceptibility factors contributed by the parent mice of the A stock. Should this hypothesis be true the observed ratios would have to be corrected for these excess susceptible animals before they could be analyzed to determine the number of dominant susceptibility factors brought into the cross by the A stock parents.

As we have seen, more than one but less than three susceptibility factors are probably contributed by these A parents. The data for response of the individuals of the (−BC) and F_2 (N×A) generations point toward the probable need of two factors. We can assume from this that these factors are *G* and *H* and that all the A stock mice carry the homozygous dominant allelomorphs while the D stock mice carry the homozygous recessive allelomorphs of these factors. By the proposed hypothesis all the A individuals would be alike while the D individuals would fall into three groups as mentioned above. Random crossing of the mice of the D stock with those of the A stock would produce susceptible F_1 (D×A) mice of two kinds, *GgHhIi* and *GgHhII*. Similarly random matings of the F_1 (D×A) mice would produce the types of F_2 (D×A) individuals shown in table 4. The types of (−BC) individuals can be worked out in the same manner. This would give us 7/151 of the susceptible F_2 (D×A) mice and 3/11 of the susceptible (−BC) mice who were susceptible to transplants of tumor 13738b only because they carried the *ii* factor complex.

Transfer these excess susceptible animals to the non-susceptible group and we get the corrected ratios shown in table 3. By this hypothesis the data for the F_2 (D×A) mice could be explained by assuming that the A stock individuals contributed 2 dominant susceptibility factors and some of the D stock animals contributed one recessive susceptibility factor. The (−BC) data fit this theory even more closely for the corrected observed ratio differs by only 0.55 times the probable error from the ratio expected when the two dominant susceptibility factors from the A stock are needed for the successful growth of tumor 13738b.

We have seen that each of these three tumors probably need two dominant susceptibility factors in the hosts that grow them successfully. The identity of the factors needed by tumor 13738b cannot be detected with these data because of the distortion of the ratios obtained with the individuals of the segregating (D×A) hybrid generations. We have but 51 F_2 (D×A) mice who received simultaneous implantations of tumors 13738a and 13738b with the ratio of reaction as follows: 15(+a+b):3(+a−b):16(−a+b):17(−a−b). It is probably safe to conclude that tumors a and b are not dependent upon the same factors for susceptibility from the A stock, for three individuals grew a without growing b. We find that

TABLE 4

Types of F_2 ($D \times A$) individuals formed when the hypothetical ii factor taken alone produces susceptibility (excess susceptible individuals are marked ++).

GAMETES OF F_1 (D×A) INDIVIDUALS	*3GHI*	*3GhI*	*3gHI*	*3ghI*	*GHi*	*gHi*	*Ghi*	*ghi*
3GHI	*9GGHHII* +	*9GGHhII* +	*9GgHHII* +	*9GgHhII* +	*3GGHHIi* +	*3GgHHIi* +	*3GGHhIi* +	*3GgHhIi* +
3GhI	*9GGHhII* +	*9GGhhII* −	*9GgHhII* +	*9GghhII* −	*3GGHhIi* +	*3GgHhIi* +	*3GGhhIi* −	*3Gghhli* −
3gHI	*9GgHHII* +	*9GgHhII* +	*9ggHHII* −	*9ggHhII* −	*3GgHHIi* +	*3ggHHIi* −	*3GgHhIi* +	*3ggHhIi* −
3ghI	*9GgHhII* +	*9Gghhll* −	*9ggHhII* −	*9gghhII* −	*3GgHhIi* +	*3ggHhIi* −	*3Gghhli* −	*3gghh Ii* −
GHi	*3GGHHIi* +	*3GGHhIi* +	*3GgHHIi* +	*3GgHhIi* +	*GGHHii* +	*GgHHii* +	*GGHhii* +	*GgHhii* +
gHi	*3GgHHIi* +	*3GgHhIi* +	*3ggHHIi* −	*3ggHhIi* −	*GgHHii* +	*ggHHii* ++	*GgHhii* +	*ggHhii* ++
Ghi	*3GGHhIi* +	*3GGhhIi* −	*3GgHhIi* +	*3GghhIi* −	*GGHhii* +	*GgHhii* +	*GGhhii* ++	*Gghhii* ++
ghi	*3GgHhIi* +	*3Gghhli* −	*3ggHhIi* −	*3gghhIi* −	*GgHhii* +	*ggHhii* ++	*Gghhii* ++	*gghhii* ++

tumors 13738c and 13738b when implanted simultaneously into 60 F_2 (D×A) mice gave a reaction of 31(+b+c):6(+b−c):5(−b+c):18 (−b−c). Here we can conclude that tumors b and c are probably not dependent upon the same susceptibility factors from the A stock mice.

TABLE 5

Theoretical ratios expected for from one to four susceptibility factors when two different tumors (a and c) are both transplanted into all of the members of large F_2 and (−BC) populations.

FACTORS	GROWTH FACTORS NEEDED	RATIO NUMBER	GENERATION	+c+a	+c−a	−c+a	−c−a	TOTAL
	a=*N*		F_2	9	0	3	4	16
	c=*NP*	1	(−BC)	1	0	1	2	4
2								
	a=*N*		F_2	9	3	3	1	16
	c=*P*	2	(−BC)	1	1	1	1	4
..								
	a=*N*		F_2	27	9	21	7	64
	c=*OP*	3	(−BC)	1	1	3	3	8
	a=*MN*		F_2	27	9	9	19	64
	c=*NP*	4	(−BC)	1	1	1	5	8
3								
	a=*N*		F_2	27	0	21	16	64
	c=*NOP*	5	(−BC)	1	0	3	4	8
	a=*NO*		F_2	27	0	9	28	64
	c=*NOP*	6	(−BC)	1	0	1	6	8
..								
	a=*M*		F_2	81	0	111	64	256
	c=*MNOP*	7	(−BC)	1	0	7	8	16
	a=*M*		F_2	81	27	111	37	256
	c=*NOP*	8	(−BC)	1	1	7	7	16
	a=*MN*		F_2	81	0	63	112	256
	c−*MNOP*	9	(−BC)	1	0	3	12	16
4								
	a=*LMN*		F_2	81	63	27	85	256
	c=*NP*	10	(−BC)	1	3	1	11	16
	a=*MN*		F_2	81	63	63	49	256
	c=*OP*	11	(−BC)	1	3	3	9	16
	a=*MNO*		F_2	81	0	27	148	256
	c=*MNOP*	12	(−BC)	1	0	1	14	16
	a=*MNO*		F_2	81	27	27	121	256
	c=*NOP*	13	(−BC)	1	1	1	13	16

Whether or not tumor b needs one factor in common with either a or c we cannot detect.

Where tumors a and c have been employed together in opposite sides of the same hosts we can analyze the reactions more fully than we were able to do with tumor b. The ratio obtained in the comparison of these two tumors should not be significantly different from one or more of the theoretical ratios in table 5 unless there is a case of linkage between some of the factors involved. There were 64 individuals in the F_2 (D×A) generation which received simultaneous transplants of tumors a and c (table 6). The

TABLE 6

Comparison of the susceptibility to tumors 13738a and 13738c in the same F_2 (D×A) individuals.

RATIOS FOR 64 MICE	+c+a	+c−a	−c+a	−c−a
1. Observed F_2 (D×A)	23.00±2.58	12.00±2.10	4.00±1.30	15.00±2.28
2. Expected by ratio 4 (table 5) 27:9:9:19	27.00±2.66	9.00±1.87	9.00±1.87	19.00±2.46
3. Expected by ratio 10 (table 5) 81:63:27:85	20.25±2.51	15.75±2.32	6.75±1.65	21.25±2.53
4. Difference between ratios 1 and 2	1.08×P.E.	1.06×P.E.	2.19×P.E.	1.19×P.E.
5. Difference between ratios 1 and 3	0.76×P.E.	1.20×P.E.	1.30×P.E.	1.82×P.E.

By ratio 4 (table 5) = 1 common factor a = *MN* and c = *NP*′
By ratio 10 (table 5) = 1 common factor a = *LMN* and c = *NP*

fact that each of these tumors probably needs two susceptibility factors for its continued growth is borne out by the observed ratio which is not significantly different from the expectation for a 27:9:9:19 ratio (ratio 2, table 6). The observed ratio will also fit an 81:63:27:85 ratio where tumor a needs the presence of three and tumor c needs the presence of two susceptibility factors. In either case they probably need the presence of one factor which is common to both of them. The reactions of 67 (−BC) mice to tumors a and c are shown in table 7. Here as in the F_2 (D×A) animals

TABLE 7

Comparison of the susceptibility to tumors 13738a and 13738c in the same (−BC) individuals.

RATIOS FOR 67 MICE	+c+a	+c−a	−c+a	−c−a
1. Observed (−BC)	15.00±2.30	5.00±1.45	5.00±1.45	42.00±2.66
2. Expected by ratio 4 (table 5) 1:1:1:5	8.37±1.82	8.37±1.82	8.37±1.82	41.87±2.67
3. Difference between ratios 1 and 2	2.25×P.E.	1.44×P.E.	1.44×P.E.	0.03×P.E.

By ratio 4 (table 5) = 1 common factor a = *MN* and c = *NP*.

the observed ratio is not significantly different from the expectation when each tumor needs the presence of two susceptibility factors. Both the F_2 (D×A) and (−BC) data indicate that these tumors need one factor in common and probably but two factors are needed by each tumor. Three different susceptibility factors carried by the A stock and transmitted by them to their hybrid offspring could account for all the susceptible individuals who grew implants of tumors 13738a and 13738c. We have called these susceptibility factors, *M*, *N* and *P* with *N* as the factor needed in common by both tumors. Whether or not tumor 13738b needs any of these three factors for growth we cannot detect with these data.

DISCUSSION

The differences between these three tumors has been repeatedly shown to be genetic and to obey the principles of multiple Mendelian factors. As STRONG and LITTLE (1920) and BITTNER (1931) have pointed out the host receiving the tumor tissue transplants is in all probability genetically alike on both sides of its body and the differences in the reactions of a host to transplants of two different tumors must be due to physiological differences between the tumors themselves. From this it is evident that genetic dissimilarities that exist between morphologically similar tumors, from the same gland of this female S 13738, and are demonstrated by the reactions of the hosts carrying tumor implants, are physiological differences. It may be that the somatic tissues of an individual's body are divisible into cells of different physiological genotypes that are not detectable morphologically. In the case of female S 13738 it is as though the cells of the mammary gland were of at least three physiological genotypes which belonged to the same phenotype. Since cancer starts from a single cell or a small group of cells the presence of different physiological genotypes among the cells of the mammary gland of an individual might account for several physiologically different mammary gland tumors occurring spontaneously in that individual.

It is of special interest that an inbred female could give rise to tumors which are morphologically similar but physiologically dissimilar. In the demonstration of this we see the genetic value of tumor tissue transplantation. The differences in these three tumors would certainly have remained undetected had no tumor transplantation work been attempted with them. It is also probable that only a few of these physiological differences that could be demonstrated by tumor transplantation are actually detected at present. Another interesting point that deserves emphasis is the finding that although these three tumors are physiologically different from each

other they all show an orderly genetic control of their reactions toward their hosts. This was shown when they were transplanted into animals of genetically controlled stocks and their hybrids. Since these differences are under the influence of an orderly genetic control it demonstrates beyond doubt the value of genetic analysis as a method of cancer research.

CONCLUSIONS

1. The growth of transplants of tumors that occurred spontaneously in a female of the highly inbred A stock is primarily dependent upon susceptibility factors carried in a homozygous dominant condition by individuals of the A stock.

2. Implants of the three tumors from female S 13738 each probably need the presence of two dominant susceptibility factors transmitted by the A parents to their hybrid offspring.

3. All of the same identical susceptibility factors are not needed by any two of these three tumors but transplants of tumors 13738a and 13738c probably require the presence of one factor which is needed in common by both of them.

4. It appears as though transplants of tumor 13738b could grow in the presence of either of two types of susceptibility factors. One type is the dominant susceptibility factors (probably two) from the A individuals and the other type is carried by certain of the D individuals where it gives a reaction similar to that of a recessive susceptibility factor.

5. These three morphologically similar tumors from the mammary gland of female S 13738 show different physiological characteristics when tumor transplants are compared in genetically controlled hosts and these physiological characteristics appear to be under an orderly genetic control which is specific for each tumor. Thus by transplanting these tumors we have demonstrated that they are apparently physiologically and genetically different although they arose at the same time in the same gland of a single mouse.

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THE RELATION OF CROSSING OVER TO CHROMOSOME ASSOCIATION IN ZEA-EUCHLAENA HYBRIDS

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THE PROBLEM

The problems to be considered in the present paper are concerned with the interrelations of various phases of the meiotic process. Conjugation is considered by most cytologists and geneticists to be necessary for the normal occurrence of crossing over, chiasma formation, post-diplotene chromosome association and chromosome disjunction. JANSSENS (1924), BELLING (1931), DARLINGTON (1931b), SAX (1930) and others hold the view that chiasmas are related to crossing over. DARLINGTON (1929, 1930, 1931b) has put forth the theory that chromosome association after diplotene is due to the presence of chiasmas. ANDERSON (1929) found that frequency of non-disjunction in Drosophila was inversely related to the frequency of crossing over. DOBZHANSKY (1931) has suggested that decreased frequency of crossing over in heterozygous translocations in Drosophila can be accounted for by assuming decreased frequency of conjugation in

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the regions affected. Certain of the hypotheses which have to do with the relations existing between various phases of the meiotic process have been tested experimentally. It is important that more such tests be made. The studies reported in the present paper were undertaken with such a purpose in mind.

It has been shown (EMERSON and BEADLE in press) that the maize chromosome which carries the *C* gene and others crosses over infrequently or not at all, in the *C*—*wx* region, with its homolog from either the Durango or the Florida form of annual teosinte (*Euchlaena mexicana*). By making use of this chromosome of teosinte, it is possible to study chromosome behavior at meiosis under two conditions, namely, with and without crossing over. The studies here reported show a definite relation between crossing over and chromosome association after diplotene. This relation is considered in connection with various theories that have to do with the meiotic mechanism.

MATERIAL AND METHODS

Some fifteen genes in the *C* chromosome of maize have been worked with by various investigators. MCCLINTOCK (1930) has demonstrated that the chromosome which carries the *C* group of linked genes is the ninth longest of the ten maize chromosomes. It will be referred to as chromosome 9 in the present paper. This chromosome has the spindle attachment so located that the ratio of the lengths of the two limbs of the chromosome is approximately 2:1 (MCCLINTOCK 1930 and unpublished). The genes in chromosome 9 which have been used in the course of the studies here reported are listed as follows:

y_{g2}	Yellow green seedling and plant
C	Aleurone color
s_h	Shrunken endosperm
w_x	Waxy endosperm
v_1	Virescent seedling

A genetic map of this chromosome showing the position of the above genes is shown in figure 1. The teosinte homologs of this chromosome carry normal allelomorphs of these genes.

In addition to the above mentioned genes, a reciprocal translocation involving maize chromosomes 8 and 9 has been used in the course of the studies. This translocation, known as "semisterile-2," has been studied by BURNHAM (1930) and by MCCLINTOCK (1930, 1931). Since this is the only translocation concerned in the present paper, it will be referred to merely as "translocation."

Some information as to the physical location of the genes in the ninth chromosome of maize is available. STADLER (1931) observed in a study of endosperm mosaics that the C and S_h genes were often lost (presumably by loss or injury of a part or all of the chromosome) without loss of W_x, but that loss of W_x was always accompanied by loss of C and S_h. This indicates that the spindle attachment is close to w_x. MCCLINTOCK (1930, 1931) has shown that the translocation concerned involves somewhat more than half the long limb of chromosome 9. She also demonstrated that the genes C, s_h and w_x lie in the same direction from the translocation point as does the short limb. BURNHAM has shown (unpublished data cited by MCCLINTOCK 1931) that the genes C, s_h and w_x lie in the order w_x, s_h, C from the translocation. The available evidence therefore indicates that the $C-w_x$ interval is included, at least for the most part, in the short limb of the chromosome.

FIGURE 1.—Genetic map of the ninth chromosome of maize showing location of genes referred to in the present paper.

Homologs of the ninth chromosome of maize from the Durango and Florida forms of annual teosinte are involved in the studies presented in this paper. For convenience in technic and for other reasons which will be apparent later, these teosinte chromosomes were transferred to maize-like stocks by repeated backcrossing of the hybrids to maize. The plants used for study were from the third and fourth backcross generations. Since the teosinte chromosomes were marked only by the genes c, S_h and W_x, it is probable that any sections outside the marked region would, provided crossing over with the maize homolog occurs, be replaced by sections from maize chromosomes. Evidence to be presented suggests that the short limb of the teosinte chromosomes was retained. For convenience, the chromosomes which are made up of at least a certain section from teosinte will be referred to as teosinte chromosomes.

Genetic studies of crossing over were made using the derived teosinte chromosomes.

For cytological observations the maize translocation was introduced for the purpose of providing a physical marker for the chromosomes which it was desired to study.

Preparations for cytological observation were made by the aceto-carmine method. Material was killed in a mixture of 7 parts alcohol and 3 parts acetic acid. Preparations were best when material was left in this

killer from one to several days. Sporocytes were stained and mounted either in aceto-carmine or a mixture of aceto-carmine and Ehrlich's haematoxylin as used by COOPER (COOPER and BRINK 1931). Sporocytes were flattened by gentle heating and by pressure of the cover glass as recommended by MCCLINTOCK (1929). All drawings were made with the aid of a camera lucida. Drawings of the chromosomes at diakinesis are semi-diagrammatic in that they are drawn with smooth outlines. Actually, by the methods used, diakinesis chromosomes in maize have irregular outlines.

TABLE 1

Backcross data on crossing over between maize chromosome 9 and its homolog from teosinte. The class carrying the dominant allelomorph of the first gene listed in column 1 is listed first, in all cases, under crossover class.

CONSTITUTION OF MAIZE CHROMOSOME	SOURCE OF TEOSINTE CHROMOSOME	CROSSOVERS IN REGION					TOTAL
		0	1	2	3	1, 3	
$y_{g2} w_x$	Durango	156 102	0 0 0 percent				258
y_{g2} s_h w_x	Durango	105 75	0 0 0 percent	0 0 0 percent			180 438
y_{g2} s_h w_x	Florida	550 475	3 0 0.3 percent	1 0 0.1 percent			1029
C w_x translocation	Florida	37 37	0 0 0 percent	2 1 3.9 percent			77
C translocation	Florida	83 122	11 25 14.9 percent				241 318
Total crossing over in *C*-translocation (equivalent to w_x translocation) interval—12.3 percent.							
C s_h w_x v_1	Durango	356 435	2 0 0.4 percent	0 0 0 percent	69 53 13.4 percent	2 0	917
Crossing over in region 3 based only on *c* S_h W_x class—10.9 percent.							
C sh w_x v_1	Florida	367 547	0 0 0 percent	0 0 0 percent	40 36 7.7 percent	0 0	990
Crossing over in region 3 based only on *c* S_h W_x class—6.2 percent.							

GENETIC STUDIES OF CROSSING OVER

Crosses of plants of the constitution $\frac{+\ +\ +\ \text{(teosinte)}}{y_{g2}\ C\ w_x\ \text{(maize)}}$ or $\frac{+\ +\ +\ \text{(teosinte)}}{y_{g2}\ s_h\ w_x\ \text{(maize)}}$ by maize, recessive for the genes concerned, are presented in table 1. It is evident from these data that there is little or no crossing over in the $y_{g2}-C$ interval. It has already been shown (EMERSON and BEADLE in press) that there is little or no crossing over in the $C-w_x$ interval in such crosses. The teosinte chromosomes from both Durango and Florida forms show similar behavior in the $y_{g2}-w_x$ interval in these crosses.

Data from the cross $\frac{++\qquad +\ \text{(teosinte)}}{C\ w_x\ \text{translocation (maize)}}$ or $\frac{+\qquad +\ \text{(teosinte)}}{C\ \text{translocation (maize)}}$ by $c\ w_x$ maize are presented in table 1. Only the teosinte chromosome from the Florida form has been studied in this interval. As compared with crossing over in the w_x-translocation interval in pure maize (12 percent based on 1305 individuals—BURNHAM 1930 and unpublished), crossing over between the maize and teosinte chromosomes occurs with approximately normal frequency in this interval. Considering the history of the teosinte chromosome, it is probable that both chromosomes in part of this interval are made up of maize chromosome segments. There must have been at least some crossing over in this interval between the original teosinte chromosome and its maize homolog. It is not possible to say with what frequency it occurred.

Data from crosses of plants of the constitution $\frac{++++\ \text{(teosinte)}}{C\ s_h\ w_x\ v_1\ \text{(maize)}}$ by $c\ s_h\ w_x\ v_1$ maize are summarized in table 1. In these crosses, classification for v_1 was somewhat difficult in the $C\ s_h\ w_x$ class but was easily made in the $c\ S_h\ W_x$ class. The crossover value based on the $c\ S_h\ W_x$ class is therefore probably more reliable than that based on both classes. Both the Durango and Florida teosinte homologs of the ninth maize chromosome were studied in these crosses. The data show that crossing over occurs in the w_x-v_1 interval with about the same frequency as between two maize chromosomes (control value 8.3 percent based on 1104 individuals).

In the data summarized in table 1, a few apparent crossovers in the $y_{g2}-s_h$, $C-s_h$ and s_h-w_x regions are recorded. Such infrequently occurring crossover types have been found in previous experiments (EMERSON and BEADLE in press). All of these which have been tested have been shown not to be due to crossing over. The cases recorded in the present paper were

not tested. They may have been the result of crossing over, contaminations or hetero-fertilization (SPRAGUE 1929).

The data indicate that crossing over between the ninth chromosome of maize and its teosinte homologs does not occur or occurs rarely in the short limb of the chromosome. Crossing over in the long limb probably occurs with about the same frequency as that observed in maize. The relation of frequency and distribution of crossing over in this chromosome to number

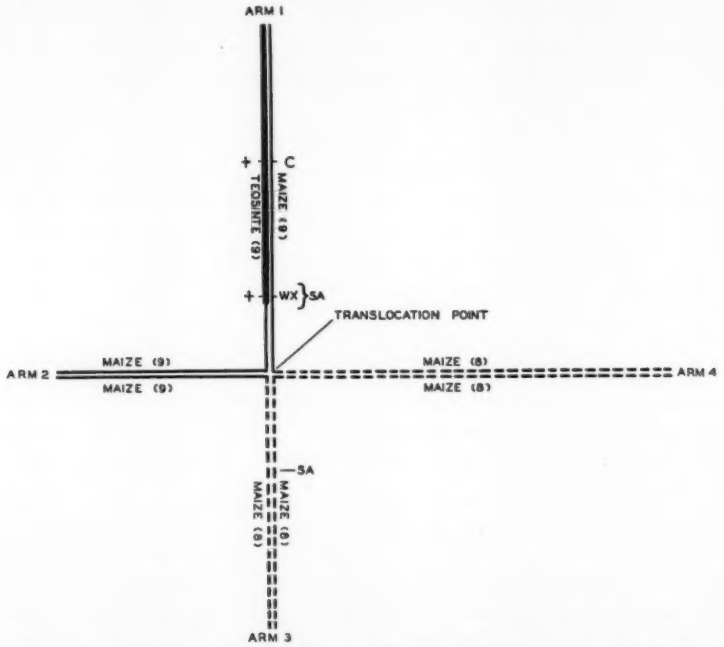

FIGURE 2.—Diagram of the set-up used for cytological observation. The portion of the non-translocated ninth chromosome known to have been derived from teosinte and known not to cross over with its corresponding maize section, is represented by a heavy line. Position of spindle attachments is indicated (SA). Lengths of the chromosomes are based on cytological determinations by MCCLINTOCK (1930).

and distribution of chiasmas and to association after diplotene will be considered later.

CYTOLOGICAL OBSERVATIONS

Using ninth chromosome homologs from Durango and from Florida teosinte in combination with translocated maize chromosomes, plants of

the constitution represented in figure 2 were obtained. Cytological observations on such plants were made for the purpose of studying pachytene pairing and post-diplotene association in arm 1 of the complex. It is this arm in which crossing over has just been considered. Pure maize plants heterozygous for the translocation were used as a control. In the control used for a study of the points of association at diakinesis in arm 1 of the complex, the longest translocated chromosome was marked with a deep-staining terminal knob (McCLINTOCK 1930). This knob provided a marker for this arm.

Pachytene chromosome association

Pachytene chromosome association in maize plants heterozygous for the translocation has been described by McCLINTOCK (1930). Parasynapsis of homologous parts takes place resulting in a cross-shaped configuration at pachytene such as is diagrammed in figure 2.

When the section of teosinte chromosome which does not cross over with its homologous maize parts is put into the complex, pachytene pairing in arm 1 of the complex is usually normal. Fourteen such cross-shaped figures were observed in complexes including a section of Durango teosinte chromosome. Two figures of such complexes in diplotene were observed. Of the complexes involving the Florida teosinte chromosome, fourteen pachytene cross-shaped figures were observed. Since with both teosinte chromosomes the results were the same, the two cases can be discussed together. Of the twenty-eight pachytene figures, 23 (11 Durango, 12 Florida) showed closely paired threads in all parts that could be followed. In these figures, no unpaired threads which might have been connected with the complex were observed. Numerous other pachytene figures were observed in which the translocation cross could not be definitely identified but in which threads throughout the nucleus could be traced and shown to be associated in pairs. Five pachytene translocation figures (3 Durango, 2 Florida) showed lack of pairing of threads in at least one arm of the cross. In one, part of one arm had apparently been torn off in the preparation of the slide. The remainder of the two threads of this arm were unpaired. Two figures showed a terminal portion (about one third) of one arm, which could not be identified, to be unpaired. One figure showed unpaired threads in approximately the median third of one arm. Another figure showed the segment beyond the spindle attachment of one arm to be unpaired (figure 3a). In this same figure, a portion of a second arm was unpaired.

From a consideration of relative lengths of the arms of the cross and from positions of the spindle attachments (McCLINTOCK 1930), the $C-s_h-w_s$

carrying arm could be identified in five figures and in all showed apparently normal pairing.

Of the two diplotene figures, one showed apparent association in all four arms of the cross. The other (figure 3b) showed the threads distal to the spindle attachment of what was probably the $C-s_h-w_x$ arm to be unpaired.

In none of the pachytene figures was reverse conjugation observed, such as McClintock (in press) has shown to be characteristic of at least two inversions in maize.

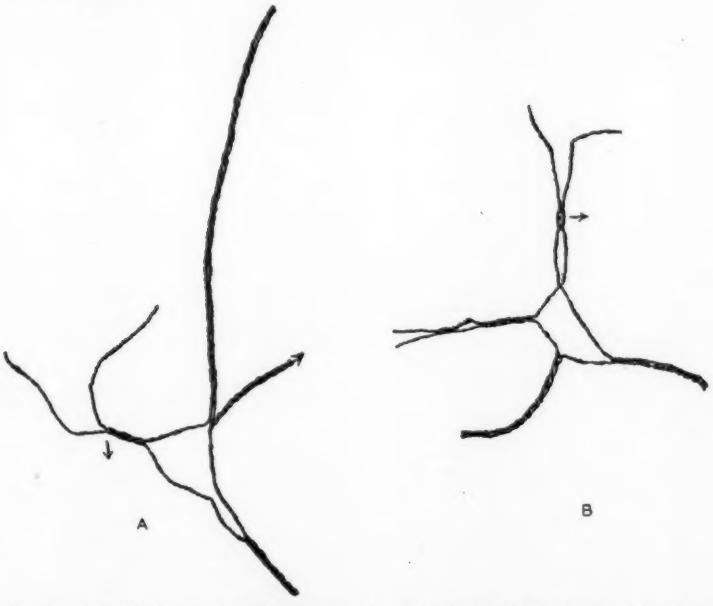

Figure 3.—A, Pachytene translocation complex including a Florida teosinte chromosome. Probable location of the spindle attachment of one chromosome is indicated by the lower arrow. B, Diplotene (early) translocation complex including a Durango teosinte chromosome. The spindle attachment in arm 1 of the complex is indicated by the arrow. Both figures A and B are semidiagrammatic. ×1565.

Diakinesis and metaphase chromosome association

As shown by the observations summarized in table 2, the heterozygous translocation in pure maize results in a ring configuration at diakinesis and metaphase in about 96 percent of the sporocytes. Diakinesis and metaphase observations agree as well as might be expected. Of 133 rings classi-

TABLE 2

Chromosome configurations of heterozygous translocation complexes in normal maize and in maize-teosinte hybrids.

ORIGIN OF NON-TRANSLOCATED CHROMOSOME 9	NUMBER OF PLANTS STUDIED	STAGE	OBSERVED FIGURES						PERCENT RINGS OF 4
			RING OF 4	CHAIN OF 4	TWO "PAIRS"	CHAIN OF 3 AND UNIVALENT	ONE "PAIR" AND 2 UNIVALENTS	TOTAL	
Maize	2	Diakinesis	235	7	0	0	0	242	97
		Metaphase	110	8	0	0	0	118	93
								360	96
Durango Teosinte	4	Diakinesis	46	183	4	4	0	237	19
		Metaphase	4	54	6	0	0	64	6
								301	17
Florida Teosinte	4	Diakinesis	7	37	156	6	2	208	3
		Metaphase	6	32	113	2	0	153	4
								361	4
Florida Teosinte	1	Diakinesis	9	25	2	0	0	36	25
		Metaphase	1	9	0	0	0	10	10
								46	22

fied at diakinesis with respect to the point of association in arm 1 (figure 2) of the translocation complex, 106 were associated only at the end of the arm (figure 4d, e). The remainder of the figures (27 or 20 percent) showed association at a point somewhere in the median region of non-translocated chromosome 9. The point of association was judged by its position to be in the region between the spindle attachment and the translocation point in arm 1 of the complex. In these figures, the chromosomes were also associated at the end of arm 1 of the complex. In most cases the chromosomes appeared to lie more or less parallel between these extreme points of association but in two figures they were opened out between the two points of association to form a figure 8 (figure 4f, g).

When the teosinte chromosome carrying the section which does not cross over with its maize homolog is put in the complex, the diakinesis and metaphase configurations are strikingly different from those observed in pure maize. Somewhat different results were obtained with the two forms of teosinte. The complex including a Durango teosinte chromosome gave

ring configurations in 17 percent of the observed cells. The difference between diakinesis and metaphase counts is probably not significant. The diakinesis determinations are easier to make and therefore are probably more reliable. The observed configurations were predominantly chains of 4 at diakinesis and metaphase (table 2). Of 42 chains classified with respect to the position of the longest chromosome of the complex, 41 showed the longest chromosome in a terminal position (figure 4a, b). This shows that either arm 1 or 4 of the complex has opened out to form the chain. Since these plants represented the fourth backcross to maize, non-translocated chromosome 8 almost certainly is a maize chromosome in some of the plants from which counts are recorded. Arm 4 of the complex, being

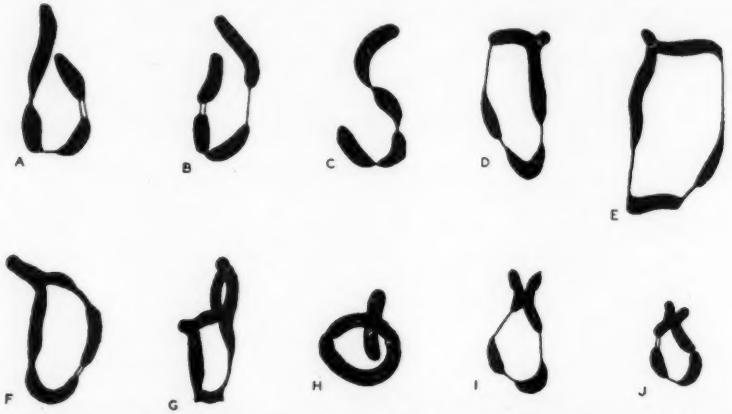

FIGURE 4.—Diakinesis figures of translocation complexes. ×1565. Semidiagrammatic. A, B, Chain of 4 including a Durango teosinte chromosome. C, Chain of 4 including a Florida teosinte chromosome. D, E, Ring of 4 in pure maize control showing end association in arm 1 of the complex which is marked by a terminal knob. F, G, Ring of 4 in maize control showing interstitial association in arm 1 of the complex. H, I, J, Ring complexes, including a Durango teosinte chromosome, showing interstitial association without end association in arm 1 of the complex.

made up of maize chromosome segments, should show association at diakinesis and metaphase similar to that observed in the pure maize control. Arm 1 of the pachytene cross which includes the $y_{v2}-w_x$ region must be the one which opens out to form a chain. A careful study was made of the rings in the plants which included a Durango teosinte chromosome in the translocation complex. The chromosome segments in arm 1 of the complex were usually (38 out of 45 figures) associated only at a point in the median portion of non-translocated chromosome 9 (figure 4h, i, j). The points of association, as far as one could determine, were within the region of arm 1

which lies between the spindle attachment and the translocation point. Seven figures showed association of segments in arm 1 of the complex at the ends only. The significance of the region of association will be discussed later.

The translocation complex in four plants with a Florida teosinte chromosome gave ring configurations in 4 percent of the figures (table 2). In contrast to the situation where a Durango teosinte chromosome was put into the complex, the configurations were predominantly two "pairs" (table 2). The members of each "pair" were different in size and were attached only

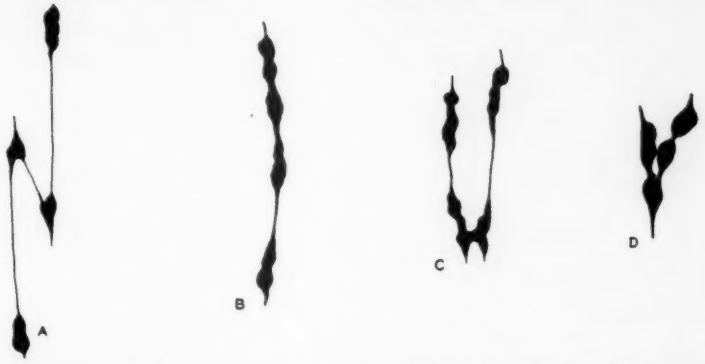

FIGURE 5.—Metaphase translocation chains of 4 including a Durango teosinte chromosome. ×1565. A, Adjacent chromosomes going to opposite poles. B, C, Two types of distribution in which adjacent chromosomes go to the same pole. D, Type of arrangement which may give 3-1 distribution of chromosomes.

at one end at diakinesis (figure 6a). The smaller member of one "pair" was about equal in size to the larger member of the other "pair." During metaphase the members of these two "pairs" were separated to a greater extent than were the members of the other pairs in the same figure (figure 6b), presumably due to the fact that they were attached at only one end. One of 7 plants studied with a Florida teosinte chromosome in the complex gave predominantly chains of 4 (table 2) as did the Durango hybrids. The longest chromosome of the complex occupied a terminal position in the chain (figure 4c). Association in arm 1 of the complex, when it occurred, was mainly confined to the region about median to non-translocated chromosome 9. As in the Durango hybrids, opening out of the complex into a chain must have been the result of non-association in arm 1 of the complex.

In the complexes which gave two "pairs," arm 1 of the complex must have been one of those in which the segments separated. Arm 3 of the com-

plex, then, must have been the other arm in which the segments separated. The reason for non-association of segments in arm 3 of the complex after diplotene is not clear. A section of teosinte chromosome may have been present in this arm though this is improbable if independent segregation of chromosomes in the original hybrid is assumed. A modified maize chromosome segment might have been introduced into arm 3 during the process of backcrossing to maize.

POLLEN STERILITY

Counts show that the heterozygous translocation in pure maize results in about 57 percent of visibly aborted pollen (BURNHAM 1930). Three plants, including in the translocation complex a Durango teosinte chromosome, showed 52 percent of aborted pollen (total number of pollen grains

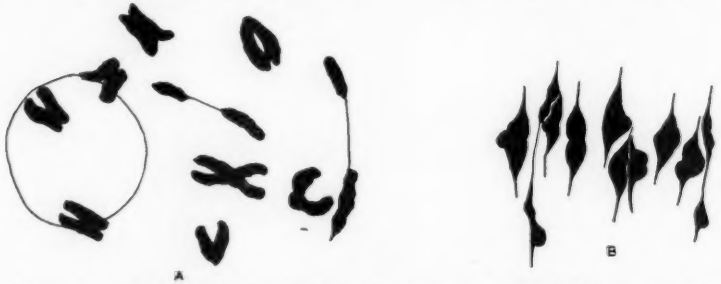

FIGURE 6.—A, Diakinesis showing translocation complex, including a Florida teosinte chromosome, broken up into two "pairs." B, Metaphase showing translocation complex, including a Florida teosinte chromosome, broken up into two "pairs."

counted—1909). Three plants of similar constitution but including a Florida teosinte chromosome gave 57 percent of aborted pollen (total number of pollen grains counted—2084). The three plants including the Florida chromosome were of the group which gave mainly two "pairs" from the translocation complex. The relation of amount of pollen sterility to chromosome disjunction will be discussed later.

DISCUSSION

Cause of reduction in crossing over

The genetic data show that crossing over does not occur or occurs rarely in the $y_{g2}-w_x$ interval, between the teosinte and maize chromosomes. It is possible that crossovers occur which lead to the formation of inviable spores. This would be expected if inversions were present. If such crossovers occur in the region under study, one should observe an amount of sterility equal to the frequency of crossing over. In the Durango hybrids

there is a small amount of visible pollen abortion but probably no more than in normal maize stocks. The ears are of such a structure that it is difficult to estimate female sterility. In the Florida hybrids, a fair estimate of female sterility can be made. It is very low, probably not significantly higher than in pure maize. If any crossovers occur between the sections of teosinte and maize chromosomes under discussion, they must be rare.

The cytological observations do not show why the $y_{g2}-w_z$ section of the ninth chromosome of maize crosses over infrequently or not at all with its corresponding section of the teosinte chromosomes. The reduction cannot be due to lack of conjugation. This means that conjugation need not necessarily result in crossing over even where other chromosomes and other parts of the same chromosome show normal crossing over.

In Drosophila many crossover reducers are known to be inverted sections (STURTEVANT 1931a). Cytological observations on teosinte-maize hybrids give no evidence of inversions in the region under study. For two inversions in maize MCCLINTOCK (in press) has shown that reverse conjugation of the inverted section with its normal homologous section frequently occurs. However, other inversions, especially short ones, may not show reverse conjugation frequently enough to be detected cytologically. The inverted sections might well be mechanically pulled parallel and therefore appear conjugated.

Gene differences between the maize and teosinte chromosomes in the $y_{g2}-w_z$ section might perhaps account for the absence of crossing over. Such a gene difference, on SAX'S theory of crossing over, might be expected to determine opening out a diplotene in the reductional plane only, which would result in no chiasmas and no crossovers.

Relation of crossing over to chromosome association and disjunction

For the purpose of the present discussion, five phases of the meiotic process are of interest. These are conjugation, chiasma formation, post-diplotene chromosome association, crossing over and chromosome disjunction.

Conjugation is the process of pairing of the chromosome threads as observed at zygotene. It is necessary for crossing over, chiasma formation, association of chromosomes after diplotene and normal disjunction of homologous chromosomes.

Theories of post-diplotene chromosome association

Two hypotheses to account for post-diplotene chromosome association will be considered. BELLING (1927) has postulated the existence of genes

which have to do with association of chromosomes after diplotene in forms which show association of homologous chromosomes at the ends. This assumes special properties of chromosome ends which cause homologous ends to remain together after the non-terminal regions have separated at diplotene.

DARLINGTON (1929, 1930, 1931b) assumes that chiasmas (exchanges of partners among paired chromatids) are responsible for post-diplotene chromosome association. If chiasmas remain constant or nearly so in number and position through diakinesis and metaphase, then it is clear from cytological observations that these chiasmas might well be the only factors responsible for chromosome association at such stages. However, in forms in which the chromosomes are associated only or mainly at the ends at diakinesis and metaphase, as is the case in maize, the apparent difficulties of this hypothesis become greater. DARLINGTON assumes that the chiasmas have been reduced in number in such cases by a process of terminalization. The terminalized chiasmas are the agents responsible for the end association of homologous chromosomes. This hypothesis offers no satisfactory explanation of why the chiasmas stop at the ends of the chromosomes instead of slipping completely off and leaving the ends unassociated.

Assuming that something other than chiasmas holds chromosome ends together after terminalization is complete, chiasmas would still seem to be a prerequisite to such association. Cytological observations indicate that ends of homologous chromosomes are no more frequently associated at diplotene than are other points of a chromosome pair (see DARLINGTON 1931b for references to literature). In forms which show end associations at later stages, the simplest interpretation is that the ends are brought together after diplotene by terminalizing chiasmas. Doctor JACK SCHULTZ of this laboratory has suggested that the matrix which is known to surround the chromonemas at diakinesis in many forms (SANDS 1923, KAUFMANN 1926, KUWADA 1927, BABCOCK and CLAUSEN 1929 and others) may be concerned in end association of chromosomes after terminalization of chiasmas. If the matrix appears before terminalization is complete, then after terminalization the matrices which had been separate will become continuous. Final separation of the chromosomes would occur by attenuation and breakage of the matrix material between the two homologs. On this view, the chiasmas could completely terminalize and would not themselves hold the chromosome ends together but would be responsible for connecting homologous ends by making the matrix continuous from one homolog to the other. KUWADA (1927) shows a photograph (plate 2, figure 8) of an end association at metaphase in Tradescantia which is sug-

gestive of the above view. The chromonemas are entirely separate but the matrix appears to be continuous between the homologous ends.

Theories of crossing over

The three current theories of crossing over all agree in assuming a relation between chiasmas and crossing over. JANSSENS' (1924) chiasmatype theory as supported by DARLINGTON (1931b) assumes that for no apparent reason, two of four chromatids undergo crossing over sometime after conjugation and prior to diplotene. BELLING'S (1931) hypothesis provides a mechanism for the process of crossing over. Both of these hypotheses assume that every chiasma is a result of a crossover between two of four chromatids. SAX (1930) assumes, as have many earlier workers (see SAX for references), that chiasmas arise by opening out in different planes at diplotene. On SAX'S theory, crossovers occur by breaking of chiasmas subsequent to the time of their formation by opening out at diplotene. On the basis of the first two hypotheses, if post-diplotene association is the result of chiasma formation, it follows that crossing over is a necessary prerequisite to such association. SAX'S hypothesis is not necessarily inconsistent with DARLINGTON'S contention that chiasmas are responsible for association after diplotene. One must simply assume that not all chiasmas break. Those which do not break can be assumed to be responsible for the persistence of chromosome association. Chromosome association without crossing over, such as is known in the male Drosophila, is possible on such a view whereas it is difficult to reconcile with the view that chiasmas arise only by crossing over and that chiasmas occasion chromosome association after diplotene.

Crossing over and post-diplotene chromosome association

In discussing the data presented in this paper with respect to its bearing on the interrelations of conjugation, chiasma formation, post-diplotene chromosome association, crossing over and chromosome disjunction, it is only necessary to consider arm 1 (figure 2) of the translocation complex. Conjugation takes place in this arm. Let us consider, first, the relation between crossing over and association of chromosomes after diplotene. The data show a definite relation between the two. When crossing over is reduced, association at diakinesis and metaphase is likewise reduced. Crossing over occurs only in the w_z-translocation interval (except possibly at the distal end of the arm beyond y_{g2}). Chromosome association is limited to the region which, so far as can be determined, coincides with the interval in which crossing over is known to occur. Both crossing over and association

of chromosomes in this region are approximately equal in frequency to corresponding determinations in the control. The agreement between crossing over and chromosome association constitutes definite evidence that the two are related.

Chiasmas and post-diplotene association of chromosomes

There is no direct cytological evidence that the associations observed in the present study between homologous chromosome segments were chiasmas. However, there is cytological evidence in many forms which indicates that interstitial points of association between homologous chromosomes during and after diplotene are chiasmas (see BELLING 1931 and DARLINGTON 1930, 1931b for discussion and references to literature). Wherever the methods have been adequate, such interstitial points of association have been shown to be real chiasmas. The interstitial nodes or points of association in maize are therefore assumed to be chiasmas. If so, it follows that chiasmas are related to both crossing over and post-diplotene association of chromosomes.

DARLINGTON (1930) has studied frequency of association of whole chromosomes and of fragments in *Fritillaria imperialis*. He found that frequency of association during and after diplotene was approximately proportional to length of chromosome. This relation is offered as evidence in favor of his hypothesis that association after diplotene is occasioned by chiasmas. The case by no means constitutes a critical test of the theory. The observed result could equally well be predicted on the assumption that association is due to something other than chiasmas and is reduced in frequency in short fragments because of reduced frequency of conjugation. Pachytene pairing was not studied in the case of the fragments.

Chiasmas and end association of chromosomes

The relation between interstitial chiasmas and chromosome association has been discussed. The bearing of the observations presented above on the relation of chiasmas and end associations of homologous chromosomes will now be considered. The ends of chromosome segments in arm 1 of the translocation complex including a teosinte chromosome were not often associated during diakinesis and metaphase. There was some crossing over and chiasma formation in this arm but both were, for the most part, limited to the region between the spindle attachment and the translocation point. The spindle attachment presumably prevents movement of chiasmas through it (argument by analogy with Drosophila where the first meiotic division is almost certainly reductional for the spindle at-

tachment [BRIDGES and ANDERSON 1925, ANDERSON 1925, MORGAN 1925, ANDERSON 1929, REDFIELD 1930, RHOADES 1931 and STURTEVANT 1931b]). Cytological observations on the method of conjugation and opening out of translocation complexes in maize (MCCLINTOCK 1930, COOPER and BRINK 1931) show that opening out occurs in the reductional plane at the translocation point. Chiasmas would therefore be unable to terminalize past such a point (see also DARLINGTON 1931a). In the translocation complex discussed in this paper, the chiasmas in arm 1 between the spindle attachment and the translocation point are unable to terminalize. Since there is little or no crossing over between the spindle attachment and the end of the short limb of the chromosome, few or no chiasmas would be expected in this region. If chiasmas are necessary for end association, few or no such associations would be observed. The data are consistent with this expectation. This does not necessarily mean that end associations are chiasmas. It was suggested above that terminalizing chiasmas may result in a continuous matrix between homologous ends and thereby be only indirectly responsible for end associations.

Bearing of the data on theories of crossing over

The data on relation of crossing over and chromosome association are not inconsistent with any of the three theories of crossing over outlined above. Either JANSSENS' or BELLING'S theory, assuming chiasmas to be responsible for post-diplotene association of chromosomes, leads to a definite relation between crossing over and frequency of association of chromosomes. Since each chiasma is the result of a crossover between two of four chromatids, short segments of chromosomes should be associated after diplotene with a frequency equal to twice the frequency of crossing over. The genetic data indicate that the crossovers in the w_x-translocation interval in the complexes including a teosinte chromosome are about as frequent as in pure maize. The control value is about 12 percent (BURNHAM 1930 and unpublished). The frequency of association of homologous segments at diakinesis (the most reliable determination) in the same arm of the complex was about 20 percent. The frequency of association expected from the crossover data is 24 percent. The agreement is fairly good.

As pointed out above, SAX'S theory can be reconciled with the view that chiasmas are responsible for chromosome association after diplotene. If put on this basis, this theory does not require a definite relation between frequency of crossing over and frequency of association. The fact that a fair agreement was found between observed association and the expectation on JANSSENS' or BELLING'S hypotheses does not disprove SAX'S

theory. On SAX's hypothesis, if chiasmas are formed between two points where opening out occurs in the reductional plane, at least two must be formed. Both need not break, in which case an agreement with the observations discussed above, though improbable, is possible.

Association and disjunction of chromosomes

The reciprocal 8–9 translocation in maize which was made use of in the present study results in about 57 percent of aborted pollen (BURNHAM 1930). MCCLINTOCK (1930) has shown that there are formed the six possible types of spores expected from random distribution of the four chromosomes in the ring. The amount of sterility indicates, however, that they are not formed in the frequencies expected on random distribution. MCCLINTOCK assumes that adjacent chromosomes go to the same pole in approximately half the cases. This accounts for the observed sterility.

The translocation complexes which were broken up into a chain of 4 by the introduction of a teosinte chromosome gave 52 percent aborted pollen. Metaphase figures were observed (figure 5) which showed that the six possible types of spores were formed. As in the ring of 4, adjacent (or end) chromosomes in the chain must go to the same pole in approximately half the cases. The slightly lower sterility may be due to there being more 3–1 distributions in the chain than in the ring (figure 5d). In the translocation complex broken up into two "pairs" the sterility was approximately the same as in the case of the ring of 4 (57.0 and 56.9 percent respectively). The possible types of spores are here reduced from six to four. The expected sterility is 50 percent since the four types of spores should be formed in equal numbers, two fertile and two sterile. The observed increase over the expected 50 percent may or may not be significant.

If, as has been argued, the breaking up of the translocation complex into two "pairs" is the result of non-association of segments in arms 1 and 3 (figure 2) then homologous spindle attachments and segments of both chromosomes 8 and 9 must undergo non-disjunction in about 50 percent of the meiotic divisions. Homologous spindle attachments are distributed approximately at random with respect to each other. This shows clearly that spindle attachment homology, in itself, need have nothing to do with chromosome disjunction. A similar conclusion was arrived at by DOBZHANSKY and STURTEVANT (1931) in a study of translocations in Drosophila. The relation of crossing over to association and disjunction of chromosomes is clear in this translocation complex which breaks up into two "pairs." ANDERSON (1929), DOBZHANSKY (1932), and REDFIELD (unpublished) have shown that chromosomes which cross over with high (nor-

mal) frequency usually go to opposite poles at the first meiotic division whereas chromosomes which undergo little or no crossing over frequently go to the same pole (non-disjunction). GOWEN (1928, 1931) has reported a case in Drosophila in which absence of crossing over in the female is accompanied by a high frequency of non-disjunction of all chromosomes. The facts presented in the present paper make it clear that reduced crossing over and high non-disjunction are not necessarily due to reduced frequency of conjugation of the chromosome segments concerned. The Drosophila data are entirely consistent with the view that normal disjunction is dependent on post-diplotene association of chromosomes which is dependent on chiasma formation which, in turn, is related (either as cause or effect) to crossing over.

SUMMARY

Crossing over in the $y_{g2}-w_x$ interval (about 45 units) between chromosome 9 of maize and its homologs from Durango and Florida forms of annual teosinte occurs infrequently or not at all. Crossing over occurs in the w_x-v_1 interval (about 8 units long) in the hybrids. Since the spindle attachment is probably close to w_x, it is assumed that crossing over between maize and teosinte chromosome 9 is confined to the long limb.

Lack of crossing over in the shorter limb between maize and teosinte chromosome 9 cannot be accounted for by lack of conjugation. It is concluded from this that conjugation or apparent conjugation does not necessarily lead to crossing over even in a form which shows normal crossing over in other chromosomes and other parts of the same chromosome. The reduction in crossing over may be due to a segment or segments of the short limb of chromosome 9 being inverted in one species with respect to the arrangment in the other. There is no direct cytological evidence in favor of this assumption. The reduction in crossing over may be due to general gene differences in this region which are not accompanied by differences in sequence. Such differences might inhibit crossing over without observably affecting conjugation.

In arm 1 (figure 2) of the translocation complex including a chromosome from teosinte, crossing over has been shown genetically to be mainly confined to the region near the translocation point. Association of chromosomes after diplotene in this arm of the complex is mainly confined to the region in which there is crossing over, presumably because chiasmas in this region cannot terminalize. Furthermore, this association is about equal to twice the frequency of crossing over. This relation supports DARLINGTON'S

hypothesis that chiasmas are responsible for post-diplotene chromosome association.

None of the three theories of crossing over considered are necessarily inconsistent with the data reported.

A case of independent assortment of homologous spindle attachments and chromosome segments at meiosis is reported. It is concluded that spindle attachment homology, in itself, has nothing to do with chromosome disjunction.

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